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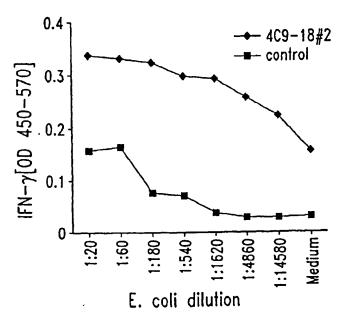
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[Continued on next page]

(54) Title: COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION



(57) Abstract: Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a *Chlamydia* antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.

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# COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION

#### **TECHNICAL FIELD**

The present invention relates generally to the detection and treatment of Chlamydial infection. In particular, the invention is related to polypeptides comprising a *Chlamydia* antigen and the use of such polypeptides for the serodiagnosis and treatment of Chlamydial infection.

#### BACKGROUND OF THE INVENTION

Chlamydiae are intracellular bacterial pathogens that are responsible for a wide variety of important human and animal infections. Chlamydia trachomatis is one of the most common causes of sexually transmitted diseases and can lead to pelvic inflammatory disease (PID), resulting in tubal obstruction and infertility. Chlamydia trachomatis may also play a role in male infertility. In 1990, the cost of treating PID in the US was estimated to be \$4 billion. Trachoma, due to ocular infection with Chlamydia trachomatis, is the leading cause of preventable blindness worldwide. Chlamydia pneumonia is a major cause of acute respiratory tract infections in humans and is also believed to play a role in the pathogenesis of atherosclerosis and, in particular, coronary heart disease. Individuals with a high titer of antibodies to Chlamydia pneumonia have been shown to be at least twice as likely to suffer from coronary heart disease as seronegative individuals. Chlamydial infections thus constitute a significant health problem both in the US and worldwide.

Chlamydial infection is often asymptomatic. For example, by the time a woman seeks medical attention for PID, irreversible damage may have already occurred resulting in infertility. There thus remains a need in the art for improved vaccines and pharmaceutical compositions for the prevention and treatment of *Chlamydia* infections. The present invention fulfills this need and further provides other related advantages.

#### SUMMARY OF THE INVENTION

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The present invention provides compositions and methods for the diagnosis and therapy of *Chlamydia* infection. In one aspect, the present invention provides polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, or a variant of such an antigen. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments,, the polypeptide comprises an amino acid

sequence encoded by a polynucleotide sequence selected from the group consisting of (a) a sequence of SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290; (b) the complements of said sequences; and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions. In specific embodiments, the polypeptides of the present invention comprise at least a portion of a *Chlamydial* protein that includes an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO: 5-14, 17-20, 26, 28, 30-32, 34, 39-43, 65, 89-109, 138-158, 167, 168, 224-262, 246, 247, 254-256, 292, 294-305 and variants thereof.

The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of a *Chlamydial* protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

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In a related aspect, polynucleotide sequences encoding the above polypeptides, recombinant expression vectors comprising one or more of these polynucleotide sequences and host cells transformed or transfected with such expression vectors are also provided.

In another aspect, the present invention provides fusion proteins comprising an inventive polypeptide, or, alternatively, an inventive polypeptide and a known *Chlamydia* antigen, as well as polynucleotides encoding such fusion proteins, in combination with a physiologically acceptable carrier or immunostimulant for use as pharmaceutical compositions and vaccines thereof.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody, both polyclonal and monoclonal, or antigen-binding fragment thereof that specifically binds to a *Chlamydial* protein; and (b) a physiologically acceptable carrier. Within other aspects, the present invention provides pharmaceutical compositions that comprise one or more *Chlamydia* polypeptides disclosed herein, or a polynucleotide molecule encoding such a polypeptide, and a physiologically acceptable carrier. The invention also provides vaccines for prophylactic and therapeutic purposes comprising one or more of the disclosed polypeptides and an immunostimulant, as defined herein, together with vaccines comprising one or more polynucleotide sequences encoding such polypeptides and an immunostimulant.

In yet another aspect, methods are provided for inducing protective immunity in a patient, comprising administering to a patient an effective amount of one or more of the above pharmaceutical compositions or vaccines.

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In yet a further aspect, methods for the treatment of Chlamydia infection in a patient are provided, the methods comprising obtaining peripheral blood mononuclear cells (PBMC) from the patient, incubating the PBMC with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated T cells and administering the incubated T cells to the patient. The present invention additionally provides methods for the treatment of Chlamydia infection that comprise incubating antigen presenting cells with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated antigen presenting cells and administering the incubated antigen presenting cells to the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient. In certain embodiments, the antigen presenting cells are selected from the group consisting of dendritic cells, macrophages, monocytes, B-cells, and fibroblasts. Compositions for the treatment of Chlamydia infection comprising T cells or antigen presenting cells that have been incubated with a polypeptide or polynucleotide of the present invention are also provided. Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

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The present invention further provides, within other aspects, methods for removing *Chlamydial*-infected cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a *Chlamydial* protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of *Chlamydial* infection in a patient, comprising administering to a patient a biological sample treated as described above. In further aspects of the subject invention, methods and diagnostic kits are provided for detecting *Chlamydia* infection in a patient. In one embodiment, the method comprises: (a) contacting a biological sample with at least one of the polypeptides or fusion proteins disclosed herein; and (b) detecting in the sample the presence of binding agents that bind to the polypeptide or fusion protein, thereby detecting *Chlamydia* infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. In one embodiment, the diagnostic kits comprise one or more of the polypeptides or fusion proteins disclosed herein in combination with a detection reagent. In yet another embodiment, the diagnostic kits comprise either a monoclonal antibody or a polyclonal antibody that binds with a polypeptide of the present invention.

The present invention also provides methods for detecting *Chlamydia* infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, at least one of the oligonucleotide primers being specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers. In one embodiment, the oligonucleotide primer comprises at least about 10 contiguous nucleotides of a polynucleotide sequence peptide disclosed herein, or of a sequence that hybridizes thereto.

In a further aspect, the present invention provides a method for detecting Chlamydia infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe. In one embodiment, the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence disclosed herein, or a sequence that hybridizes thereto.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

#### SEQUENCE IDENTIFIERS

SEQ ID NO: 1 is the determined DNA sequence for the C. trachomatis clone 1-B1-66.

SEQ ID NO: 2 is the determined DNA sequence for the C. trachomatis clone 4-D7-28.

SEQ ID NO: 3 is the determined DNA sequence for the C. trachomatis clone 3-G3-10.

SEQ ID NO: 4 is the determined DNA sequence for the C. trachomatis 30 clone 10-C10-31.

SEQ ID NO: 5 is the predicted amino acid sequence for 1-B1-66.

SEQ ID NO: 6 is the predicted amino acid sequence for 4-D7-28.

SEQ ID NO: 7 is a first predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 8 is a second predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 9 is a third predicted amino acid sequence for 3-G3-10.

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SEO ID NO: 10 is a	fourth predicted	amino acid seque	nce for 3-G3-10.

SEO ID NO: 11 is a fifth predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 12 is the predicted amino acid sequence for 10-C10-31.

SEQ ID NO: 13 is the amino acid sequence of the synthetic peptide 1-

5 B1-66/48-67.

CtC7.8-12

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SEQ ID NO: 14 is the amino acid sequence of the synthetic peptide 1-B1-66/58-77.

SEQ ID NO: 15 is the determined DNA sequence for the *C. trachomatis* serovar LGV II clone 2C7-8

SEQ ID NO: 16 is a DNA sequence of a putative open reading frame from a region of the C. trachomatis serovar D genome to which 2C7-8 maps

SEQ ID NO: 17 is the predicted amino acid sequence encoded by the DNA sequence of SEQ ID NO: 16

SEQ ID NO: 18 is the amino acid sequence of the synthetic peptide

SEQ ID NO: 19 is the amino acid sequence of the synthetic peptide CtC7.8-13

SEQ ID NO: 20 is the predicted amino acid sequence encoded by a second putative open reading from C. trachomatis serovar D

SEQ ID NO: 21 is the determined DNA sequence for clone 4C9-18 from C. trachomatis LGV II

SEQ ID NO: 22 is the determined DNA sequence homologous to Lipoamide Dehydrogenase from C. trachomatis LGV II

SEQ ID NO: 23 is the determined DNA sequence homologous to Hypothetical protein from C. trachomatis LGV II

SEQ ID NO: 24 is the determined DNA sequence homologous to Ubiquinone Mehtyltransferase from C. trachomatis LGV II

SEQ ID NO: 25 is the determined DNA sequence for clone 4C9-18#2 BL21 pLysS from C. trachomatis LGV II

SEQ ID NO: 26 is the predicted amino acid sequence for 4C9-18#2 from C. trachomatis LGV II

SEQ ID NO: 27 is the determined DNA sequence for Cp-SWIB from C. pneumonia strain TWAR

SEQ ID NO: 28 is the predicted amino acid sequence for Cp-SWIB from C. pneumonia strain TWAR

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SEQ II	D NO: 29 is the determined DNA sequence for Cp-S13 from C.
pneumonia strain TW	AR
SEQ II	D NO: 30 is the predicted amino acid sequence for Cp-S13 from
C. pneumonia strain 7	WAR
SEQ I	D NO: 31 is the amino acid sequence for a 10mer consensus
peptide from CtC7.8-	12 and CtC7.8-13
SEQ I	D NO: 32 is the predicted amino acid sequence for clone 2C7-8
from C. trachomatis	LGV II
	D NO: 33 is the DNA sequence corresponding to nucleotides
597304-597145 of th	ne C. trachomatis serovar D genome (NCBI, BLASTN search),
which shows homolog	
SEQ I	D NO: 34 is the predicted amino acid sequence encoded by the
sequence of SEQ ID	NO: 33
SEQ I	D NO: 35 is the DNA sequence for C.p. SWIB Nde (5' primer)
from C. pneumonia	
SEQ I	D NO: 36 is the DNA sequence for C.p. SWIB EcoRI (3' primer)
from C. pneumonia	
SEQ I	D NO: 37 is the DNA sequence for C.p. S13 Nde (5' primer) from
C. pneumonia	
SEQ 1	ID NO: 38 is the DNA sequence for C.p. S13 EcoRI (3' primer)
from C. pneumonia	
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SEQ ID NO: 39 is the amino acid sequence for CtSwib 52-67 peptide from C. trachomatis LGV II

SEQ ID NO: 40 is the amino acid sequence for CpSwib 53-68 peptide from C. pneumonia

SEQ ID NO: 41 is the amino acid sequence for HuSwib 288-302 peptide from Human SWI domain

SEQ ID NO: 42 is the amino acid sequence for CtSWI-T 822-837 peptide from the topoisomerase-SWIB fusion of C. trachomatis

SEQ ID NO: 43 is the amino acid sequence for CpSWI-T 828-842 peptide from the topoisomerase-SWIB fusion of C. pneumonia

SEQ ID NO: 44 is a first determined DNA sequence for the C. trachomatis LGV II clone 19783.3 jen.seq(1>509)CTL2#11-3', representing the 3' end.

SEQ ID NO: 45 is a second determined DNA sequence for the C. trachomatis LGV II clone 19783.4, jen.seq(1>481)CTL2#11-5', representing the 5' end. 35

SEQ ID NO: 46 is the determined DNA sequence for the *C. trachomatis* LGV II clone19784CTL2\_12consensus.seq(1>427)CTL2#12.

SEQ ID NO: 47 is the determined DNA sequence for the C. trachomatis LGV II clone 19785.4,jen.seq(1>600)CTL2#16-5', representing the 5' end.

SEQ ID NO: 48 is a first determined DNA sequence for the C. trachomatis LGV II clone 19786.3, jen. seq(1>600) CTL2#18-3', representing the 3' end.

SEQ ID NO: 49 is a second determined DNA sequence for the C. trachomatis LGV II clone 19786.4 jen.seq(1>600)CTL2#18-5', representing the 5' end.

SEQ ID NO: 50 is the determined DNA sequence for the C. trachomatis

0 LGV II clone 19788CTL2\_21consensus.seq(1>406)CTL2#21.

SEQ ID NO: 51 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19790CTL2\_23consensus.seq(1>602)CTL2#23.

SEQ ID NO: 52 is the determined DNA sequence for the C. trachomatis LGV II clone 19791CTL2\_24consensus.seq(1>145)CTL2#24.

SEQ ID NO: 53 is the determined DNA sequence for the *C. trachomatis* LGV II clone CTL2#4.

SEQ ID NO: 54 is the determined DNA sequence for the C. trachomatis LGV II clone CTL2#8b.

SEQ ID NO: 55 is the determined DNA sequence for the *C. trachomatis* 20 LGV II clone15-G1-89, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 56 is the determined DNA sequence for the C. trachomatis LGV II clone 14-H1-4, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 57 is the determined DNA sequence for the C. trachomatis LGV II clone 12-G3-83, sharing homology to the hypothetical protein CT622.

SEQ ID NO: 58 is the determined DNA sequence for the *C. trachomatis* LGV II clone 12-B3-95, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 59 is the determined DNA sequence for the *C. trachomatis* 30 LGV II clone 11-H4-28, sharing homology to the dnaK gene CT396.

SEQ ID NO: 60 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-H3-68, sharing partial homology to the PGP6-D virulence protein and L1 ribosomal gene CT318.

SEQ ID NO: 61 is the determined DNA sequence for the C. trachomatis

LGV II clone 11-G1-34, sharing partial homology to the malate dehydrogenase gene

CT376 and to the glycogen hydrolase gene CT042.

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SEQ ID NO: 62 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-G10-46, sharing homology to the hypothetical protein CT610.

SEQ ID NO: 63 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-C12-91, sharing homology to the OMP2 gene CT443.

SEQ ID NO: 64 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-A3-93, sharing homology to the HAD superfamily gene CT103.

SEQ ID NO: 65 is the determined amino acid sequence for the C. trachomatis LGV II clone 14-H1-4, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 66 is the determined DNA sequence for the C. trachomatis LGV II clone CtL2#9.

SEQ ID NO: 67 is the determined DNA sequence for the C. trachomatis LGV II clone CtL2#7.

SEQ ID NO: 68 is the determined DNA sequence for the C. trachomatis
15 LGV II clone CtL2#6.

SEQ ID NO: 69 is the determined DNA sequence for the C. trachomatis LGV II clone CtL2#5.

SEQ ID NO: 70 is the determined DNA sequence for the C. trachomatis LGV II clone CtL2#2.

SEQ ID NO: 71 is the determined DNA sequence for the C. trachomatis LGV II clone CtL2#1.

SEQ ID NO: 72 is a first determined DNA sequence for the C. trachomatis LGV II clone 23509.2CtL2#3-5', representing the 5' end.

SEQ ID NO: 73 is a second determined DNA sequence for the C. trachomatis LGV II clone 23509.1CtL2#3-3', representing the 3' end.

SEQ ID NO: 74 is a first determined DNA sequence for the C. trachomatis LGV II clone 22121.2CtL2#10-5', representing the 5' end.

SEQ ID NO: 75 is a second determined DNA sequence for the C. trachomatis LGV II clone 22121.1CtL2#10-3', representing the 3' end.

SEQ ID NO: 76 is the determined DNA sequence for the C. trachomatis LGV II clone 19787.6CtL2#19-5', representing the 5' end.

SEQ ID NO: 77 is the determined DNA sequence for the *C. pneumoniae* LGV II clone CpS13-His.

SEQ ID NO: 78 is the determined DNA sequence for the C. pneumoniae 35 LGV II clone Cp SWIB-His.

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SEQ ID NO: 79 is the determined DNA sequence for the C. trachomatis LGV II clone 23-G7-68, sharing partial homology to the L11, L10 and L1 ribosomal protein.

SEQ ID NO: 80 is the determined DNA sequence for the *C. trachomatis*5 LGV II clone 22-F8-91, sharing homology to the pmpC gene.

SEQ ID NO: 81 is the determined DNA sequence for the *C. trachomatis* LGV II clone 21-E8-95, sharing homology to the CT610-CT613 genes.

SEQ ID NO: 82 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19-F12-57, sharing homology to the CT858 and recA genes.

SEQ ID NO: 83 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19-F12-53, sharing homology to the CT445 gene encoding glutamyl tRNA synthetase.

SEQ ID NO: 84 is the determined DNA sequence for the C. trachomatis LGV II clone 19-A5-54, sharing homology to the cryptic plasmid gene.

SEQ ID NO: 85 is the determined DNA sequence for the C. trachomatis LGV II clone 17-E11-72, sharing partial homology to the OppC\_2 and pmpD genes.

SEQ ID NO: 86 is the determined DNA sequence for the C. trachomatis LGV II clone 17-C1-77, sharing partial homology to the CT857 and CT858 open reading frames.

SEQ ID NO: 87 is the determined DNA sequence for the *C. trachomatis* LGV II clone 15-H2-76, sharing partial homology to the pmpD and SycE genes, and to the CT089 ORF.

SEQ ID NO: 88 is the determined DNA sequence for the C. trachomatis LGV II clone 15-A3-26, sharing homology to the CT858 ORF.

SEQ ID NO: 89 is the determined amino acid sequence for the C. pnuemoniae clone Cp\_SWIB-His.

SEQ ID NO: 90 is the determined amino acid sequence for the C. trachomatis LGV II clone CtL2\_LPDA\_FL.

SEQ ID NO: 91 is the determined amino acid sequence for the C. 30 pnuemoniae clone CpS13-His.

SEQ ID NO: 92 is the determined amino acid sequence for the C. trachomatis LGV II clone CtL2\_TSA\_FL.

SEQ ID NO: 93 is the amino acid sequence for Ct-Swib 43-61 peptide from C. trachomatis LGV II.

SEQ ID NO: 94 is the amino acid sequence for Ct-Swib 48-67 peptide from C. trachomatis LGV II.

- SEQ ID NO: 95 is the amino acid sequence for Ct-Swib 52-71 peptide from C. trachomatis LGV II.
- SEQ ID NO: 96 is the amino acid sequence for Ct-Swib 58-77 peptide from C. trachomatis LGV II.
- SEQ ID NO: 97 is the amino acid sequence for Ct-Swib 63-82 peptide from C. trachomatis LGV II.

- SEQ ID NO: 98 is the amino acid sequence for Ct-Swib 51-66 peptide from C. trachomatis LGV II.
- SEQ ID NO: 99 is the amino acid sequence for Cp-Swib 52-67 peptide from C. pneumonia.
  - SEQ ID NO: 100 is the amino acid sequence for Cp-Swib 37-51 peptide from C. pneumonia.
  - SEQ ID NO: 101 is the amino acid sequence for Cp-Swib 32-51 peptide from C. pneumonia.
- SEQ ID NO: 102 is the amino acid sequence for Cp-Swib 37-56 peptide 15 from C. pneumonia.
  - SEQ ID NO: 103 is the amino acid sequence for Ct-Swib 36-50 peptide from C. trachomatis.
- SEQ ID NO: 104 is the amino acid sequence for Ct-S13 46-65 peptide from C. trachomatis. 20
  - SEQ ID NO: 105 is the amino acid sequence for Ct-S13 60-80 peptide from C. trachomatis.
  - SEQ ID NO: 106 is the amino acid sequence for Ct-S13 1-20 peptide from C. trachomatis.
- SEQ ID NO: 107 is the amino acid sequence for Ct-S13 46-65 peptide 25 from C. trachomatis.
  - SEQ ID NO: 108 is the amino acid sequence for Ct-S13 56-75 peptide from C. trachomatis.
- SEQ ID NO: 109 is the amino acid sequence for Cp-S13 56-75 peptide from C. pneumoniae.
  - SEQ ID NO: 110 is the determined DNA sequence for the C. trachomatis LGV II clone 21-G12-60, containing partial open reading frames for hypothetical proteins CT875, CT229 and CT228.
- SEQ ID NO: 111 is the determined DNA sequence for the C. trachomatis LGV II clone 22-B3-53, sharing homology to the CT110 ORF of GroEL.

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SEQ ID NO: 112 is the determined DNA sequence for the C. trachomatis LGV II clone 22-A1-49, sharing partial homology to the CT660 and CT659 ORFs.

SEQ ID NO: 113 is the determined DNA sequence for the C. trachomatis LGV II clone 17-E2-9, sharing partial homology to the CT611 and CT 610 ORFs.

SEQ ID NO: 114 is the determined DNA sequence for the C. trachomatis LGV II clone 17-C10-31, sharing partial homology to the CT858 ORF.

SEQ ID NO: 115 is the determined DNA sequence for the C. 10 trachomatis LGV II clone 21-C7-66, sharing homology to the dnaK-like gene.

SEQ ID NO: 116 is the determined DNA sequence for the C. trachomatis LGV II clone 20-G3-45, containing part of the pmpB gene CT413.

SEQ ID NO: 117 is the determined DNA sequence for the C. trachomatis LGV II clone 18-C5-2, sharing homology to the S1 ribosomal protein ORF.

SEQ ID NO: 118 is the determined DNA sequence for the C. trachomatis LGV II clone 17-C5-19, containing part of the ORFs for CT431 and CT430.

SEQ ID NO: 119 is the determined DNA sequence for the C. trachomatis LGV II clone 16-D4-22, contains partial sequences of ORF3 and ORF4 of the plasmid for growth within mammalian cells.

SEQ ID NO: 120 is the determined full-length DNA sequence for the C. trachomatis serovar LGV II Cap1 gene CT529.

SEQ ID NO: 121 is the predicted full-length amino acid sequence for the C. trachomatis serovar LGV II Cap1 gene CT529.

SEQ ID NO: 122 is the determined full-length DNA sequence for the C. trachomatis serovar E Capl gene CT529.

SEQ ID NO: 123 is the predicted full-length amino acid sequence for the C. trachomatis serovar E Cap1 gene CT529.

SEQ ID NO: 124 is the determined full-length DNA sequence for the C.

30 trachomatis serovar 1A Cap1 gene CT529.

SEQ ID NO: 125 is the predicted full-length amino acid sequence for the C. trachomatis serovar 1A Cap1 gene CT529.

SEQ ID NO: 126 is the determined full-length DNA sequence for the C. trachomatis serovar G Cap1 gene CT529.

35 SEQ ID NO: 127 is the predicted full-length amino acid sequence for the C. trachomatis serovar G Cap1 gene CT529.

SEQ ID NO: 128 is the determined full-length DNA sequence for the C. trachomatis serovar F1 NII Cap1 gene CT529.

SEQ ID NO: 129 is the predicted full-length amino acid sequence for the C. trachomatis serovar F1 NII Cap1 gene CT529.

SEO ID NO: 130 is the determined full-length DNA sequence for the C. trachomatis serovar L1 Cap1 gene CT529.

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SEQ ID NO: 131 is the predicted full-length amino acid sequence for the C. trachomatis serovar L1 Cap1 gene CT529.

SEQ ID NO: 132 is the determined full-length DNA sequence for the C. trachomatis serovar L3 Cap1 gene CT529. 10

SEQ ID NO: 133 is the predicted full-length amino acid sequence for the C. trachomatis serovar L3 Cap1 gene CT529.

SEQ ID NO: 134 is the determined full-length DNA sequence for the C. trachomatis serovar Ba Capl gene CT529.

SEQ ID NO: 135 is the predicted full-length amino acid sequence for the 15 C. trachomatis serovar Ba Capl gene CT529.

SEQ ID NO: 136 is the determined full-length DNA sequence for the C. trachomatis serovar MOPN Capl gene CT529.

SEQ ID NO: 137 is the predicted full-length amino acid sequence for the C. trachomatis serovar MOPN Cap1 gene CT529. 20

SEQ ID NO: 138 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #124-139 of C. trachomatis serovar L2.

SEQ ID NO: 139 is the determined amino acid sequence for the Capl CT529 ORF peptide #132-147 of C. trachomatis serovar L2.

SEQ ID NO: 140 is the determined amino acid sequence for the Cap1 25 CT529 ORF peptide #138-155 of C. trachomatis serovar L2.

SEQ ID NO: 141 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #146-163 of C. trachomatis serovar L2.

SEQ ID NO: 142 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #154-171 of C. trachomatis serovar L2.

SEQ ID NO: 143 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #162-178 of C. trachomatis serovar L2.

SEQ ID NO: 144 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-147 of C. trachomatis serovar L2.

SEQ ID NO: 145 is the determined amino acid sequence for the Capl 35 CT529 ORF peptide #139-147 of C. trachomatis serovar L2.

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SEQ ID NO: 146 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #140-147 of C. trachomatis serovar L2.

SEQ ID NO: 147 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-146 of C. trachomatis serovar L2.

SEQ ID NO: 148 is the determined amino acid sequence for the Capl CT529 ORF peptide #138-145 of C. trachomatis serovar L2.

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SEQ ID NO: 149 is the determined amino acid sequence for the Cap1 CT529 ORF peptide # F140->I of C. trachomatis serovar L2.

SEQ ID NO: 150 is the determined amino acid sequence for the Capl CT529 ORF peptide ##S139>Ga of C. trachomatis serovar L2.

SEQ ID NO: 151 is the determined amino acid sequence for the Capl CT529 ORF peptide ##S139>Gb of C. trachomatis serovar L2.

SEQ ID NO: 152 is the determined amino acid sequence for the peptide # 2 C7.8-6 of the 216aa ORF of C. trachomatis serovar L2.

15 SEQ ID NO: 153 is the determined amino acid sequence for the peptide #2 C7.8-7 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 154 is the determined amino acid sequence for the peptide # 2 C7.8-8 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 155 is the determined amino acid sequence for the peptide 20 #2 C7.8-9 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 156 is the determined amino acid sequence for the peptide #2 C7.8-10 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 157 is the determined amino acid sequence for the 53 amino acid residue peptide of the 216aa ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 158 is the determined amino acid sequence for the 52 amino acid residue peptide of the CT529 ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 159 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 160 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 161 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 for serovars other than L2 and MOPN.

35 SEQ ID NO: 162 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovars other than L2 and MOPN.

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SEQ ID NO: 163 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 164 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 165 is the determined DNA sequence for the 5' (forward) primer for pBIB-KS.

SEQ ID NO: 166 is the determined DNA sequence for the 5' (reverse) primer for pBIB-KS.

SEQ ID NO: 167 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar L2.

SEQ ID NO: 168 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar D.

SEQ ID NO: 169 is the determined full-length DNA sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 170 is the determined full-length DNA sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 171 is the determined full-length DNA sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 172 is the determined full-length DNA sequence for the C. 20 trachomatis pmpD gene.

SEQ ID NO: 173 is the determined full-length DNA sequence for the C. trachomatis pmpC gene.

SEQ ID NO: 174 is the determined full-length DNA sequence for the C. trachomatis pmpB gene.

SEQ ID NO: 175 is the predicted full-length amino acid sequence for the *C. trachomatis* pmpI gene.

SEQ ID NO: 176 is the predicted full-length amino acid sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 177 is the predicted full-length amino acid sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 178 is the predicted full-length amino acid sequence for the C. trachomatis pmpD gene.

SEQ ID NO: 179 is the predicted full-length amino acid sequence for the  $\it C.\ trachomatis$  pmpC gene.

SEQ ID NO: 180 is the predicted full-length amino acid sequence for the C. trachomatis pmpB gene.

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SEQ ID NO: 181 is the determined DNA sequence minus the signal sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 182 is a subsequently determined full-length DNA sequence for the C. trachomatis pmpG gene:

SEQ ID NO: 183 is the determined DNA sequence minus the signal sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 184 is a first determined DNA sequence representing the carboxy terminus for the C. trachomatis pmpD gene.

SEQ ID NO: 185 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the *C. trachomatis* pmpD gene.

SEQ ID NO: 186 is a first determined DNA sequence representing the carboxy terminus for the C. trachomatis pmpC gene.

SEQ ID NO: 187 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the *C. trachomatis* pmpC gene.

SEQ ID NO: 188 is the determined DNA sequence representing the C. pneumoniae serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 189 is the predicted amino acid sequence minus the signal sequence for the *C. trachomatis* pmpI gene.

SEQ ID NO: 190 is subsequently predicted amino acid sequence for the 20 C. trachomatis pmpG gene.

SEQ ID NO: 191 is the predicted amino acid sequence minus the signal sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 192 is a first predicted amino acid sequence representing the carboxy terminus for the C. trachomatis pmpD gene.

SEQ ID NO: 193 is a second predicted amino acid sequence representing the Amino terminus minus the signal sequence for the *C. trachomatis* pmpD gene.

SEQ ID NO: 194 is a first predicted amino acid sequence representing the Carboxy terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 195 is a second predicted amino acid sequence representing the Amino terminus for the C. trachomatis pmpC gene.

SEQ ID NO: 196 is the predicted amino acid sequence representing the C. pneumoniae serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 197 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpC gene in the SKB vaccine vector.

SEQ ID NO: 198 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpC gene in the SKB vaccine vector.

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SEQ ID NO: 199 is the determined DNA sequence for the insertion sequence for cloning the C. trachomatis pmpC gene in the SKB vaccine vector.

SEQ ID NO: 200 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpD gene in the SKB vaccine vector.

SEQ ID NO: 201 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpD gene in the SKB vaccine vector.

SEQ ID NO: 202 is the determined DNA sequence for the insertion sequence for cloning the C. trachomatis pmpD gene in the SKB vaccine vector.

SEQ ID NO: 203 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpE gene in the SKB vaccine vector.

SEQ ID NO: 204 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpE gene in the SKB vaccine vector.

SEQ ID NO: 205 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpG gene in the SKB vaccine vector.

SEQ ID NO: 206 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpG gene in the SKB vaccine vector.

SEQ ID NO: 207 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the C. trachomatis pmpC gene in the pET17b vector.

SEQ ID NO: 208 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 209 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the C. trachomatis pmpC gene in the pET17b vector.

SEQ ID NO: 210 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the C. trachomatis pmpC gene in the pET17b vector.

SEQ ID NO: 211 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 212 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

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SEQ ID NO: 213 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 214 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the C. trachomatis pmpD gene in the pET17b vector.

SEQ ID NO: 215 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpE gene in the pET17b vector.

SEQ ID NO: 216 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 217 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpE gene in the pET17b vector.

SEQ ID NO: 218 is the amino acid sequence for the insertion sequence for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 219 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpG gene in the pET17b vector.

SEQ ID NO: 220 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpG gene in the pET17b vector.

SEQ ID NO: 221 is the amino acid sequence for the insertion sequence 20 for cloning the *C. trachomatis* pmpG gene in the pET17b vector.

SEQ ID NO: 222 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpI gene in the pET17b vector.

SEQ ID NO: 223 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpI gene in the pET17b vector.

SEQ ID NO: 224 is the determined amino acid sequence for the C. pneumoniae Swib peptide 1-20.

SEQ ID NO: 225 is the determined amino acid sequence for the C. pneumoniae Swib peptide 6-25.

SEQ ID NO: 226 is the determined amino acid sequence for the C. 30 pneumoniae Swib peptide 12-31.

SEQ ID NO: 227 is the determined amino acid sequence for the C. pneumoniae Swib peptide 17-36.

SEQ ID NO: 228 is the determined amino acid sequence for the C. pneumoniae Swib peptide 22-41.

35 SEQ ID NO: 229 is the determined amino acid sequence for the C. pneumoniae Swib peptide 27-46.

- SEQ ID NO: 230 is the determined amino acid sequence for the C. pneumoniae Swib peptide 42-61.
- SEQ ID NO: 231 is the determined amino acid sequence for the C. pneumoniae Swib peptide 46-65.
- SEQ ID NO: 232 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 51-70.
  - SEQ ID NO: 233 is the determined amino acid sequence for the C. pneumoniae Swib peptide 56-75.
- SEQ ID NO: 234 is the determined amino acid sequence for the C. pneumoniae Swib peptide 61-80.
  - SEQ ID NO: 235 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 66-87.
  - SEQ ID NO: 236 is the determined amino acid sequence for the C. trachomatis OMCB peptide 103-122.
- SEQ ID NO: 237 is the determined amino acid sequence for the C. trachomatis OMCB peptide 108-127.
  - SEQ ID NO: 238 is the determined amino acid sequence for the C. trachomatis OMCB peptide 113-132.
- SEQ ID NO: 239 is the determined amino acid sequence for the C. 20 trachomatis OMCB peptide 118-137.
  - SEQ ID NO: 240 is the determined amino acid sequence for the C. trachomatis OMCB peptide 123-143.
  - SEQ ID NO: 241 is the determined amino acid sequence for the C. trachomatis OMCB peptide 128-147.
- 25 SEQ ID NO: 242 is the determined amino acid sequence for the C. trachomatis OMCB peptide 133-152.
  - SEQ ID NO: 243 is the determined amino acid sequence for the C. trachomatis OMCB peptide 137-156.
- SEQ ID NO: 244 is the determined amino acid sequence for the C. 30 trachomatis OMCB peptide 142-161.
  - SEQ ID NO: 245 is the determined amino acid sequence for the C. trachomatis OMCB peptide 147-166.
  - SEQ ID NO: 246 is the determined amino acid sequence for the C. trachomatis OMCB peptide 152-171.
- 35 SEQ ID NO: 247 is the determined amino acid sequence for the C. trachomatis OMCB peptide 157-176.

- SEQ ID NO: 248 is the determined amino acid sequence for the C. trachomatis OMCB peptide 162-181.
- SEQ ID NO: 249 is the determined amino acid sequence for the C. trachomatis OMCB peptide 167-186.
- 5 SEQ ID NO: 250 is the determined amino acid sequence for the C. trachomatis OMCB peptide 171-190.
  - SEQ ID NO: 251 is the determined amino acid sequence for the C. trachomatis OMCB peptide 171-186.
- SEQ ID NO: 252 is the determined amino acid sequence for the C. 10 trachomatis OMCB peptide 175-186.
  - SEQ ID NO: 252 is the determined amino acid sequence for the C. trachomatis OMCB peptide 175-186.
  - SEQ ID NO: 253 is the determined amino acid sequence for the C. pneumoniae OMCB peptide 185-198.
- SEQ ID NO: 254 is the determined amino acid sequence for the C. trachomatis TSA peptide 96-115.
  - SEQ ID NO: 255 is the determined amino acid sequence for the C. trachomatis TSA peptide 101-120.
- SEQ ID NO: 256 is the determined amino acid sequence for the C. 20 trachomatis TSA peptide 106-125.
  - SEQ ID NO: 257 is the determined amino acid sequence for the C. trachomatis TSA peptide 111-130.
  - SEQ ID NO: 258 is the determined amino acid sequence for the C. trachomatis TSA peptide 116-135.
- 25 SEQ ID NO: 259 is the determined amino acid sequence for the C. trachomatis TSA peptide 121-140.
  - SEQ ID NO: 260 is the determined amino acid sequence for the C. trachomatis TSA peptide 126-145.
- SEQ ID NO: 261 is the determined amino acid sequence for the C. 30 trachomatis TSA peptide 131-150.
  - SEQ ID NO: 262 is the determined amino acid sequence for the C. trachomatis TSA peptide 136-155.
  - SEQ ID NO: 263 is the determined full-length DNA sequence for the C. trachomatis CT529/Cap 1 gene serovar I.
- 35 SEQ ID NO: 264 is the predicted full-length amino sequence for the C. trachomatis CT529/Cap 1 gene serovar I.

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SEQ ID NO: 265 is the determined full-length DNA sequence for the C. trachomatis CT529/Cap 1 gene serovar K.

SEQ ID NO: 266 is the predicted full-length amino sequence for the C. trachomatis CT529/Cap 1 gene serovar K.

SEQ ID NO: 267 is the determined DNA sequence for the C. trachomatis clone 17-G4-36 sharing homology to part of the ORF of DNA-dirrected RNA polymerase beta subunit- CT315 in serD.

SEQ ID NO: 268 is the determined DNA sequence for the partial sequence of the C. trachomatis CT016 gene in clone 2E10.

SEQ ID NO: 269 is the determined DNA sequence for the partial sequence of the C. trachomatis tRNA syntase gene in clone 2E10.

SEQ ID NO: 270 is the determined DNA sequence for the partial sequence for the C. trachomatis clpX gene in clone 2E10.

SEQ ID NO: 271 is a first determined DNA sequence for the C. 5 trachomatis clone CtL2gam-30 representing the 5'end.

SEQ ID NO: 272 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-30 representing the 3'end.

SEQ ID NO: 273 is the determined DNA sequence for the C. trachomatis clone CtL2gam-28.

SEQ ID NO: 274 is the determined DNA sequence for the C. trachomatis clone CtL2gam-27.

SEQ ID NO: 275 is the determined DNA sequence for the C. trachomatis clone CtL2gam-26.

SEQ ID NO: 276 is the determined DNA sequence for the C. 25 trachomatis clone CtL2gam-24.

SEQ ID NO: 277 is the determined DNA sequence for the C. trachomatis clone CtL2gam-23.

SEQ ID NO: 278 is the determined DNA sequence for the C. trachomatis clone CtL2gam-21.

SEQ ID NO: 279 is the determined DNA sequence for the C. trachomatis clone CtL2gam-18.

SEQ ID NO: 280 is the determined DNA sequence for the C. trachomatis clone CtL2gam-17.

SEQ ID NO: 281 is a first determined DNA sequence for the C. trachomatis clone CtL2gam-15 representing the 5' end.

- SEQ ID NO: 282 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-15 representing the 3' end.
- SEQ ID NO: 283 is the determined DNA sequence for the C. trachomatis clone CtL2gam-13.
- SEQ ID NO: 284 is the determined DNA sequence for the C. trachomatis clone CtL2gam-10.
- SEQ ID NO: 285 is the determined DNA sequence for the C. trachomatis clone CtL2gam-8.
- SEQ ID NO: 286 is a first determined DNA sequence for the C. trachomatis clone CtL2gam-6 representing the 5' end.
  - SEQ ID NO: 287 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-6 representing the 3' end.
  - SEQ ID NO: 288 is the determined DNA sequence for the C. trachomatis clone CtL2gam-5.
- SEQ ID NO: 289 is the determined DNA sequence for the C. trachomatis clone CtL2gam-2.
  - SEQ ID NO: 290 is the determined DNA sequence for the C. trachomatis clone CtL2gam-1.
- SEQ ID NO: 291 is the determined full-length DNA sequence for the C. 20 pneumoniae homologue of the CT529 gene.
  - SEQ ID NO: 292 is the predicted full-length amino acid sequence for the *C. pneumoniae* homologue of the CT529 gene.
  - SEQ ID NO: 293 is the determined DNA sequence for the insertion sequence for cloning the C. trachomatis pmpG gene in the SKB vaccine vector.
- SEQ ID NO: 294 is the amino acid sequence of an open reading frame of clone CT603.
  - SEQ ID NO: 295 is the amino acid sequence of a first open reading frame of clone CT875.
- SEQ ID NO: 296 is the amino acid sequence of a second open reading 30 frame of clone CT875.
  - SEQ ID NO: 297 is the amino acid sequence of a first open reading frame of clone CT858.
  - SEQ ID NO: 298 is the amino acid sequence of a second open reading frame of clone CT858.
- 35 SEQ ID NO: 299 is the amino acid sequence of an open reading frame of clone CT622.

- SEQ ID NO: 300 is the amino acid sequence of an open reading frame of clone CT610.
- SEQ ID NO: 301 is the amino acid sequence of an open reading frame of clone CT396.
- 5 SEQ ID NO: 302 is the amino acid sequence of an open reading frame of clone CT318.
  - SEQ ID NO: 304 is the amino acid sequence for *C. trachomatis*, serovar L2 rCt529c1-125 having a modified N-terminal sequence (6-His tag).
- SEQ ID NO: 305 is the amino acid sequence for *C. trachomatis*, serovar 10 L2 rCt529c1-125.
  - SEQ ID NO: 306 is the sense primer used in the synthesis of the PmpA(N-term) fusion protein.
  - SEQ ID NO: 307 is the antisense primer used in the synthesis of the PmpA(N-term) fusion protein.
- SEQ ID NO: 308 is the DNA sequence encoding the PmpA(N-term) fusion protein.
  - SEQ ID NO: 309 is the amino acid sequence of the PmpA(N-term) fusion protein.
- SEQ ID NO: 310 is the sense primer used in the synthesis of the 20 PmpA(C-term) fusion protein.
  - SEQ ID NO: 311 is the antisense primer used in the synthesis of the PmpA(C-term) fusion protein.
  - SEQ ID NO: 312 is the DNA sequence encoding the PmpA(C-term) fusion protein.
- SEQ ID NO: 313 is the amino acid sequence of the PmpA(C-term) fusion protein.
  - SEQ ID NO: 314 is the sense primer used in the synthesis of the PmpF(N-term) fusion protein.
- SEQ ID NO: 315 is the antisense primer used in the synthesis of the 30 PmpF(N-term) fusion protein.
  - SEQ ID NO: 316 is the DNA sequence encoding the PmpF(N-term) fusion protein.
  - SEQ ID NO: 317 is the amino acid sequence of the PmpF(N-term) fusion protein.
- SEQ ID NO: 318 is the sense primer used in the synthesis of the PmpF(C-term) fusion protein.

SEQ ID NO: 319 is the antisense primer used in the synthesis of the PmpF(C-term) fusion protein.

SEQ ID NO: 320 is the DNA sequence encoding the PmpF(C-term) fusion protein.

5 SEQ ID NO: 321 is the amino acid sequence of the PmpF(C-term) fusion protein.

SEQ ID NO: 322 is the sense primer used in the synthesis of the PmpH(N-term) fusion protein.

SEQ ID NO: 323 is the antisense primer used in the synthesis of the 10 PmpH(N-term) fusion protein.

SEQ ID NO: 324 is the DNA sequence encoding the PmpH(N-term) fusion protein.

SEQ ID NO: 325 is the amino acid sequence of the PmpH(N-term) fusion protein.

SEQ ID NO: 326 is the sense primer used in the synthesis of the PmpH(C-term) fusion protein.

SEQ ID NO: 327 is the antisense primer used in the synthesis of the PmpH(C-term) fusion protein.

SEQ ID NO: 328 is the DNA sequence encoding the PmpH(C-term) 20 fusion protein.

SEQ ID NO: 329 is the amino acid sequence of the PmpH(C-term) fusion protein.

SEQ ID NO: 330 is the sense primer used in the synthesis of the PmpB(1) fusion protein.

SEQ ID NO: 331 is the antisense primer used in the synthesis of the PmpB(1) fusion protein.

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SEQ ID NO: 332 is the DNA sequence encoding the PmpB(1) fusion protein.

SEQ ID NO: 333 is the amino acid sequence of the PmpB(1) fusion 30 protein.

SEQ ID NO: 334 is the sense primer used in the synthesis of the PmpB(2) fusion protein.

SEQ ID NO: 335 is the antisense primer used in the synthesis of the PmpB(2) fusion protein.

35 SEQ ID NO: 336 is the DNA sequence encoding the PmpB(2) fusion protein.

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- 24 SEQ ID NO: 337 is the amino acid sequence of the PmpB(2) fusion protein. SEQ ID NO: 338 is the sense primer used in the synthesis of the PmpB(3) fusion protein. SEQ ID NO: 339 is the antisense primer used in the synthesis of the PmpB(3) fusion protein. SEQ ID NO: 340 is the DNA sequence encoding the PmpB(3) fusion protein. SEQ ID NO: 341 is the amino acid sequence of the PmpB(3) fusion protein. SEQ ID NO: 342 is the sense primer used in the synthesis of the PmpB(4) fusion protein. SEQ ID NO: 343 is the antisense primer used in the synthesis of the PmpB(4) fusion protein. SEO ID NO: 344 is the DNA sequence encoding the PmpB(4) fusion protein. SEQ ID NO: 345 is the amino acid sequence of the PmpB(4) fusion protein. SEQ ID NO: 346 is the sense primer used in the synthesis of the PmpC(1) fusion protein. SEQ ID NO: 347 is the antisense primer used in the synthesis of the PmpC(1) fusion protein. SEQ ID NO: 348 is the DNA sequence encoding the PmpC(1) fusion protein. SEQ ID NO: 349 is the amino acid sequence of the PmpC(1) fusion protein. SEQ ID NO: 350 is the sense primer used in the synthesis of the PmpC(2) fusion protein. SEQ ID NO: 351 is the antisense primer used in the synthesis of the PmpC(2) fusion protein.
  - SEQ ID NO: 352 is the DNA sequence encoding the PmpC(2) fusion protein.

    SEQ ID NO: 353 is the amino acid sequence of the PmpC(2) fusion protein.
- 35 SEQ ID NO: 354 is the sense primer used in the synthesis of the PmpC(3) fusion protein.

SEQ ID NO: 355 is the antisense primer used in the synthesis of the PmpC(3) fusion protein.

SEQ ID NO: 356 is the DNA sequence encoding the PmpC(3) fusion protein.

SEQ ID NO: 357 is the amino acid sequence of the PmpC(3) fusion protein.

#### DESCRIPTION OF THE FIGURES

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Fig. 1 illustrates induction of INF-γ from a *Chlamydia*-specific T cell line activated by target cells expressing clone 4C9-18#2.

Fig. 2 illustrates retroviral vectors pBIB-KS1,2,3 modified to contain a Kosak translation initiation site and stop codons.

Fig. 3 shows specific lysis in a chromium release assay of P815 cells pulsed with *Chlamydia* peptides CtC7.8-12 (SEQ ID NO: 18) and CtC7.8-13 (SEQ ID NO: 19).

Fig. 4 shows antibody isotype titers in C57Bl/6 mice immunized with *C. trachomatis* SWIB protein.

Fig. 5 shows *Chlamydia*-specific T-cell proliferative responses in splenocytes from C3H mice immunized with *C. trachomatis* SWIB protein.

Fig. 6 illustrates the 5' and 3' primer sequences designed from C. pneumoniae which were used to isolate the SWIB and S13 genes from C. pneumoniae.

Figs. 7A and 7B show induction of IFN-γ from a human anti-chlamydia T-cell line (TCL-8) capable of cross-reacting to C. trachomatis and C. pneumonia upon activation by monocyte-derived dendritic cells expressing chlamydial proteins.

Fig. 8 shows the identification of T cell epitopes in Chlamydial ribosomal S13 protein with T-cell line TCL 8 EB/DC.

Fig. 9 illustrates the proliferative response of CP-21 T-cells generated against C. pnuemoniae-infected dendritic cells to recombinant C. pneumonia-SWIBprotein, but not C. trachomatis SWIB protein.

Fig. 10 shows the *C. trachomatis*-specific SWIB proliferative responses of a primary T-cell line (TCT-10 EB) from an asymptomatic donor.

Fig. 11 illustrates the identification of T-cell epitope in *C. trachomatis* SWIB with an antigen specific T-cell line (TCL-10 EB).

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#### DETAILED DESCRIPTION OF THE INVENTION

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As noted above, the present invention is generally directed to compositions and methods for the diagnosis and treatment of Chlamydial infection. In one aspect, the compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a *Chlamydia* antigen, or a variant thereof.

In specific embodiments, the subject invention discloses polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, wherein the *Chlamydia* antigen comprises an amino acid sequence encoded by a polynucleotide molecule including a sequence selected from the group consisting of (a) nucleotide sequences recited in SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290 (b) the complements of said nucleotide sequences, and (c) variants of such sequences.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e., antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of one of the inventive antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *Chlamydia* antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments.

An "immunogenic portion" of an antigen is a portion that is capable of reacting with sera obtained from a *Chlamydia*-infected individual (i.e., generates an absorbance reading with sera from infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals, in a representative ELISA assay described herein). Such immunogenic portions generally comprise at least about 5 amino acid residues, more preferably at least about 10, and

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most preferably at least about 20 amino acid residues. Methods for preparing and identifying immunogenic portions of antigens of known sequence are well known in the art and include those summarized in Paul, Fundamental Immunology, 3rd ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigenspecific" if they specifically bind to an antigen (i.e., they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native Chlamydia protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (e.g., in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, 125 I-labeled Protein A.

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Examples of immunogenic portions of antigens contemplated by the present invention include, for example, the T cell stimulating epitopes provided in SEQ ID NO: 9, 10, 18, 19, 31, 39, 93-96, 98, 100-102, 106, 108, 138-140, 158, 167, 168, 246, 247 and 254-256. Polypeptides comprising at least an immunogenic portion of one or more *Chlamydia* antigens as described herein may generally be used, alone or in combination, to detect Chlamydial infection in a patient.

The compositions and methods of the present invention also encompass variants of the above polypeptides and polynucleotide molecules. Such variants include, but are not limited to, naturally occurring allelic variants of the inventive sequences. In particular, variants include other *Chlamydiae* serovars, such as serovars D, E and F, as well as the several LGV serovars which share homology to the inventive polypeptide and polynucleotide molecules described herein. Preferably, the serovar homologues show 95-99% homology to the corresponding polypeptide sequence(s) described herein.

A polypeptide "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such

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that the antigenic properties of the polypeptide are retained. In a preferred embodiment, variant polypeptides differ from an identified sequence by substitution, deletion or addition of five amino acids or fewer. Such variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the antigenic properties of the modified polypeptide using, for example, the representative procedures described herein. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (e.g., 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

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As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide. Variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example,

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a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A polynucleotide "variant" is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or additions such that the immunogenicity of the encoded polypeptide is not diminished, relative to the native protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotidedirected site-specific mutagenesis as taught, for example, by Adelman et al. (DNA, 2:183, 1983). Nucleotide variants may be naturally occurring allelic variants as discussed below, or non-naturally occurring variants. The polypeptides provided by the present invention include variants that are encoded by polynucleotide sequences which are substantially homologous to one or more of the polynucleotide sequences "Substantial homology," as used herein, refers to specifically recited herein. polynucleotide sequences that are capable of hybridizing under moderately stringent conditions. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the event of cross-species homology, at 45°C with 0.5X SSC; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Such hybridizing polynucleotide sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, encode a polypeptide that is the same as a polypeptide of the present invention.

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Two nucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

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Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Resarch Foundaiton, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) Fast and sensitive multiple sequence alignments on a microcomputer CABIOS 5:151-153; Myers, E.W. and Muller W. (1988) Optimal alignments in linear space CABIOS 4:11-17; Robinson, E.D. (1971) Comb. Theor 11:105; Santou, N. Nes, M. (1987) The neighbor joining method. A new method for reconstructing phylogenetic trees Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) Rapid similarity searches of nucleic acid and protein data banks Proc. Natl. Acad., Sci. USA 80:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) Add. APL. Math 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity methods of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. (U.S.A.) 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

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One illustrative example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1977) Nuc. Acids Res. 25:3389-3402 and Altschul et al. (1990) J. Mol. Biol. 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<a href="http://www.ncbi.nlm.nih.gov/">http://www.ncbi.nlm.nih.gov/</a>) In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix can be

used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

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Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or amino acid sequence in the comparison window may comprise additions or deletions (i.e. gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Therefore, the present invention provides polynucleotide and polypeptide sequences having substantial identity to the sequences disclosed herein, for example those comprising at least 50% or more sequence identity, preferably at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a polynucleotide or polypeptide sequence of this invention using the methods described herein, (e.g., BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two polynucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

In additional embodiments, the present invention provides isolated polynucleotides or polypeptides comprising various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides and polypeptides encompassed by this invention may comprise at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000

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or more contiguous nucleotides of one or more of the disclosed sequences, as well as all intermediate lengths therebetween. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, etc.; 21, 22, 23, etc.; 30, 31, 32, etc.; 50, 51, 52, 53, etc.; 100, 101, 102, 103, etc.; 150, 151, 152, 153, etc.; including all integers through the 200-500; 500-1,000, and the like.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative DNA segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

Also included in the scope of the present invention are alleles of the genes encoding the nucleotide sequences recited in herein. As used herein, an "allele" or "allellic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone or in combination with the others, one or more times in a given sequence. In specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a Chlamydia antigen (or a variant of such an antigen), that comprises one or more of the amino acid sequences encoded by (a) a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1-4, 15 21-25, 44-64, 66-76 and 79-88; (b) the complements of such DNA sequences or (c) DNA sequences substantially homologous to a sequence in (a) or (b). As discussed in the Examples below, several of the Chlamydia antigens disclosed herein recognize a T cell line that recognizes both Chlamydia trachomatis and Chlamydia pneumoniae infected monocyte-derived dendritic cells, indicating that they may represent an immunoreactive epitope shared by Chlamydia trachomatis and Chlamydia pneumoniae. The antigens may thus be

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employed in a vaccine for both *C. trachomatis* genital tract infections and for *C. pneumonia* infections. Further characterization of these *Chlamydia* antigens from *Chlamydia trachomatis* and *Chlamydia pneumonia* to determine the extent of cross-reactivity is provided in Example 6. Additionally, Example 4 describes cDNA fragments (SEQ ID NO: 15, 16 and 33) isolated from *C. trachomatis* which encode proteins (SEQ ID NO: 17-19 and 32) capable of stimulating a *Chlamydia*-specific murine CD8+ T cell line.

In general, Chlamydia antigens, and polynucleotide sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, polynucleotide molecules encoding Chlamydia antigens may be isolated from a Chlamydia genomic or cDNA expression library by screening with a Chlamydiaspecific T cell line as described below, and sequenced using techniques well known to those of skill in the art. Additionally, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for Chlamydia-associated expression (i.e., expression that is at least two fold greater in Chlamydia-infected cells than in controls, as determined using a representative assay provided herein). Such screens may be performed using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., Proc. Natl. Acad. Sci. USA 93:10614-10619, 1996 and Heller et al., Proc. Natl. Acad. Sci. USA 94:2150-2155, 1997). Alternatively, polypeptides may be amplified from cDNA prepared from cells expressing the proteins described herein.. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequencespecific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

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Antigens may be produced recombinantly, as described below, by inserting a polynucleotide sequence that encodes the antigen into an expression vector and expressing the antigen in an appropriate host. Antigens may be evaluated for a desired property, such as the ability to react with sera obtained from a *Chlamydia*-infected individual as described herein, and may be sequenced using, for example, traditional Edman chemistry. *See* Edman and Berg, *Eur. J. Biochem.* 80:116-132, 1967.

Polynucleotide sequences encoding antigens may also be obtained by screening an appropriate *Chlamydia* cDNA or genomic DNA library for polynucleotide sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of isolated antigens. Degenerate oligonucleotide sequences for use in such a screen may be designed and synthesized, and the screen may be performed, as described (for example) in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold

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Spring Harbor Laboratories, Cold Spring Harbor, NY (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using the above oligonucleotides in methods well known in the art, to isolate a nucleic acid probe from a cDNA or genomic library. The library screen may then be performed using the isolated probe.

An amplified portion may be used to isolate a full length gene from a suitable library (e.g., a Chlamydia cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

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For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with <sup>32</sup>P) using well known techniques. A bacterial or bacteriophage library is then screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (see Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences are then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using techniques well known in the art (see, for example, Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263, 1987; Erlich ed., PCR Technology, Stockton Press, NY, 1989), and software well known in the art may also be employed. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may

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be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (see Triglia et al., Nucl. Acids Res. 16:8186, 1988), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Additional techniques include capture PCR (Lagerstrom et al., PCR Methods Applic. 1:111-19, 1991) and walking PCR (Parker et al., Nucl. Acids. Res. 19:3055-60, 1991). Transcription-Mediated Amplification, or TMA is another method that may be utilized for the amplification of DNA, rRNA, or mRNA, as described in Patent No. PCT/US91/03184. This autocatalytic and isothermic non-PCR based method utilizes two primers and two enzymes: RNA polymerase and reverse transcriptase. One primer contains a promoter sequence for RNA polymerase. In the first amplification, the promoter-primer hybridizes to the target rRNA at a defined site. Reverse transcriptase creates a DNA copy of the target rRNA by extension from the 3'end of the promoterprimer. The RNA in the resulting complex is degraded and a second primer binds to the DNA copy. A new strand of DNA is synthesized from the end of the primer by reverse transcriptase creating double stranded DNA. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesized RNA amplicons re-enters the TMA process and serves as a template for a new round of replication leading to the expotential expansion of the RNA amplicon. Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

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In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length cDNA sequences may also be obtained by analysis of genomic fragments.

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Polynucleotide variants may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase phosphoramidite chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (see Adelman et al., DNA 2:183, 1983). Alternatively, RNA molecules may be generated by in vitro or in vivo transcription of DNA sequences encoding a Chlamydial protein, or portion thereof, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain portions may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may be administered to a patient such that the encoded polypeptide is generated in vivo (e.g., by transfecting antigen-presenting cells, such as dendritic cells, with a cDNA construct encoding a Chlamydial polypeptide, and administering the transfected cells to the patient).

A portion of a sequence complementary to a coding sequence (i.e., an antisense polynucleotide) may also be used as a probe or to modulate gene expression. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells of tissues to facilitate the production of antisense RNA. An antisense polynucleotide may be used, as described herein, to inhibit expression of a *Chlamydial* protein. Antisense technology can be used to control gene expression through triplehelix formation, which compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (see Gee et al., In Huber and Carr, Molecular and Immunologic Approaches, Futura Publishing Co. (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (e.g., promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

A portion of a coding sequence, or of a complementary sequence, may also be designed as a probe or primer to detect gene expression. Probes may be labeled with a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers, as noted above, are preferably 22-30 nucleotides in length.

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Any polynucleotide may be further modified to increase stability in vivo. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional

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bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

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Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division, Foster City, CA, and may be operated according to the manufacturer's instructions.

As noted above, immunogenic portions of *Chlamydia* antigens may be prepared and identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3d ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptide portions of the native antigen for immunogenic properties. The representative ELISAs described herein may generally be employed in these screens. An immunogenic portion of a polypeptide is a portion that, within such representative assays, generates a signal in such assays that is substantially similar to that generated by the full length antigen. In other words, an immunogenic portion of a *Chlamydia* antigen generates at least about 20%, and preferably about 100%, of the signal induced by the full length antigen in a model ELISA as described herein.

Portions and other variants of *Chlamydia* antigens may be generated by synthetic or recombinant means. Variants of a native antigen may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Sections of the polynucleotide sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

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Recombinant polypeptides containing portions and/or variants of a native antigen may be readily prepared from a polynucleotide sequence encoding the polypeptide using a variety of techniques well known to those of ordinary skill in the art. For example, supernatants from suitable host/vector systems which secrete recombinant protein into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant protein.

Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides as described herein. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a polynucleotide molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line, such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

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In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in an isolated, substantially pure, form. Preferably, the polypeptides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known *Chlamydial* protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein. A DNA sequence encoding a fusion protein of the present invention may be constructed using known recombinant DNA techniques to assemble separate DNA sequences encoding, for example, the first and second polypeptides, into an appropriate expression vector. The 3' end of a DNA

sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., Gene 40:39-46, 1985; Murphy et al., Proc. Natl. Acad. Sci. USA 83:8258-8562, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. As an alternative to the use of a peptide linker sequence (when desired), one can utilize non-essential N-terminal amino acid regions (when present) on the first and second polypeptides to separate the functional domains and prevent steric hindrance.

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The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided that comprise a polypeptide of the present invention together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (see, for example, Stoute et al. New Engl. J. Med., 336:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises

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approximately the first third of the protein (e.g., the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in E. coli (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

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In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene 43*:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology 10*:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In another embodiment, a Mycobacterium tuberculosis-derived Ra12 polynucleotide is linked to at least an immunogenic portion of a polynucleotide of this invention. Ra12 compositions and methods for their use inenhancing expression of heterologous polynucleotide sequences is described in U.S. Patent Application 60/158,585, the disclosure of which is incorporated herein by reference in its entirety. Briefly, Ra12 refers to a polynucleotide region that is a subsequence of a Mycobacterium tuberculosis MTB32A nucleic acid. MTB32A is a serine protease of 32 KD molecular weight encoded by a gene in virulent and avirulent strains of M. tuberculosis. The nucleotide sequence and amino acid sequence of MTB32A have been described (U.S. Patent Application 60/158,585; see also, Skeiky et al., Infection and Immun. (1999) 67:3998-4007, incorporated herein by reference. In one embodiment, the Ra12 polypeptide used in the production of fusion polypeptides comprises a C-terminal fragment of the MTB32A coding sequence that is effective for enhancing the

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expression and/or immunogenicity of heterologous Chlamydial antigenic polypeptides with which it is fused. In another embodiment, the Ra12 polypeptide corresponds to an approximately 14 kD C-terminal fragment of MTB32A comprising some or all of amino acid residues 192 to 323 of MTB32A.

Recombinant nucleic acids, which encode a fusion polypeptide comprising a Ra12 polypeptide and a heterologous Chlamydia polypeptide of interest, can be readily constructed by conventional genetic engineering techniques. Recombinant nucleic acids are constructed so that, preferably, a Ra12 polynucleotide sequence is located 5' to a selected heterologous Chlamydia polynucleotide sequence. It may also be appropriate to place a Ra12 polynucleotide sequence 3' to a selected heterologous polynucleotide sequence or to insert a heterologous polynucleotide sequence into a site within a Ra12 polynucleotide sequence.

In addition, any suitable polynucleotide that encodes a Ra12 or a portion or other variant thereof can be used in constructing recombinant fusion polynucleotides comprising Ra12 and one or more Chlamydia polynucleotides disclosed herein. Preferred Ra12 polynucleotides generally comprise at least about 15 consecutive nucleotides, at least about 30 nucleotides, at least about 100 nucleotides, at least about 200 nucleotides, or at least about 300 nucleotides that encode a portion of a Ra12 polypeptide.

Ra12 polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes a Ra12 polypeptide or a portion thereof) or may comprise a variant of such a sequence. Ra12 polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not substantially diminished, relative to a fusion polypeptide comprising a native Ra12 polypeptide. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native Ra12 polypeptide or a portion thereof.

In another aspect, the present invention provides methods for using one or more of the above polypeptides or fusion proteins (or polynucleotides encoding such polypeptides or fusion proteins) to induce protective immunity against Chlamydial infection in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with a disease, or may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat Chlamydial infection.

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In this aspect, the polypeptide, fusion protein or polynucleotide molecule is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines may comprise one or more of the above polypeptides and an immunostimulant, such as an adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other *Chlamydia* antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

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Alternatively, a vaccine may contain polynucleotides encoding one or more polypeptides or fusion proteins as described above, such that the polypeptide is generated in situ. In such vaccines, the polynucleotides may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary polynucleotide sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the polynucleotides may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve Techniques for incorporating the use of a non-pathogenic (defective) virus. polynucleotides into such expression systems are well known to those of ordinary skill in the art. The polynucleotides may also be administered as "naked" plasmid vectors as described, for example, in Ulmer et al., Science 259:1745-1749, 1993 and reviewed by Cohen, Science 259:1691-1692, 1993. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The uptake of naked

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polynucleotides may be increased by incorporating the polynucleotides into and/or onto biodegradable beads, which are efficiently transported into the cells. The preparation and use of such systems is well known in the art.

In a related aspect, a polynucleotide vaccine as described above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known *Chlamydia* antigen. For example, administration of polynucleotides encoding a polypeptide of the present invention, either "naked" or in a delivery system as described above, may be followed by administration of an antigen in order to enhance the protective immune effect of the vaccine.

Polypeptides and polynucleotides disclosed herein may also be employed in adoptive immunotherapy for the treatment of *Chlamydial* infection. Adoptive immunotherapy may be broadly classified into either active or passive immunotherapy. In active immunotherapy, treatment relies on the *in vivo* stimulation of the endogenous host immune system with the administration of immune responsemodifying agents (for example, vaccines, bacterial adjuvants, and/or cytokines).

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In passive immunotherapy, treatment involves the delivery of biologic reagents with established immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate anti-Chlamydia effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T lymphocytes (for example, CD8+ cytotoxic T-lymphocyte, CD4+ T-helper), killer cells (such as Natural Killer cells, lymphokine-activated killer cells), B cells, or antigen presenting cells (such as dendritic cells and macrophages) expressing the disclosed antigens. The polypeptides disclosed herein may also be used to generate antibodies or anti-idiotypic antibodies (as in U.S. Patent No. 4,918,164), for passive immunotherapy.

The predominant method of procuring adequate numbers of T-cells for adoptive immunotherapy is to grow immune T-cells in vitro. Culture conditions for expanding single antigen-specific T-cells to several billion in number with retention of antigen recognition in vivo are well known in the art. These in vitro culture conditions typically utilize intermittent stimulation with antigen, often in the presence of cytokines, such as IL-2, and non-dividing feeder cells. As noted above, the immunoreactive polypeptides described herein may be used to rapidly expand antigen-specific T cell cultures in order to generate sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast, or B-cells, may be pulsed with immunoreactive polypeptides, or polynucleotide sequence(s) may be introduced into antigen presenting cells, using a variety of standard techniques well known in the art. For example, antigen presenting

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cells may be transfected or transduced with a polynucleotide sequence, wherein said sequence contains a promoter region appropriate for increasing expression, and can be expressed as part of a recombinant virus or other expression system. Several viral vectors may be used to transduce an antigen presenting cell, including pox virus, vaccinia virus, and adenovirus; also, antigen presenting cells may be transfected with polynucleotide sequences disclosed herein by a variety of means, including gene-gun technology, lipid-mediated delivery, electroporation, osmotic shock, and particlate delivery mechanisms, resulting in efficient and acceptable expression levels as determined by one of ordinary skill in the art. For cultured T-cells to be effective in therapy, the cultured T-cells must be able to grow and distribute widely and to survive long term *in vivo*. Studies have demonstrated that cultured T-cells can be induced to grow *in vivo* and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (see, for example, Cheever, M., et al, "Therapy With Cultured T Cells: Principles Revisited," *Immunological Reviews*, 157:177, 1997).

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The polypeptides disclosed herein may also be employed to generate and/or isolate chlamydial-reactive T-cells, which can then be administered to the patient. In one technique, antigen-specific T-cell lines may be generated by *in vivo* immunization with short peptides corresponding to immunogenic portions of the disclosed polypeptides. The resulting antigen specific CD8+ or CD4+ T-cell clones may be isolated from the patient, expanded using standard tissue culture techniques, and returned to the patient.

Alternatively, peptides corresponding to immunogenic portions of the polypeptides may be employed to generate *Chlamydia* reactive T cell subsets by selective *in vitro* stimulation and expansion of autologous T cells to provide antigenspecific T cells which may be subsequently transferred to the patient as described, for example, by Chang *et al*, (Crit. Rev. Oncol. Hematol., 22(3), 213, 1996). Cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as Isolex<sup>TM</sup> System, available from Nexell Therapeutics, Inc. Irvine, CA. The separated cells are stimulated with one or more of the immunoreactive polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigen-specific T cells. The population of antigen-specific T cells is then expanded using standard techniques and the cells are administered back to the patient.

In other embodiments, T-cell and/or antibody receptors specific for the polypeptides disclosed herein can be cloned, expanded, and transferred into other vectors or effector cells for use in adoptive immunotherapy. In particular, T cells may

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be transfected with the appropriate genes to express the variable domains from chlamydia specific monoclonal antibodies as the extracellular recognition elements and joined to the T cell receptor signaling chains, resulting in T cell activation, specific lysis, and cytokine release. This enables the T cell to redirect its specificity in an MHC-independent manner. See for example, Eshhar, Z., Cancer Immunol Immunother, 45(3-4):131-6, 1997 and Hwu, P., et al, Cancer Res, 55(15):3369-73, 1995. Another embodiment may include the transfection of chlamydia antigen specific alpha and beta T cell receptor chains into alternate T cells, as in Cole, DJ, et al, Cancer Res, 55(4):748-52, 1995.

In a further embodiment, syngeneic or autologous dendritic cells may be pulsed with peptides corresponding to at least an immunogenic portion of a polypeptide disclosed herein. The resulting antigen-specific dendritic cells may either be transferred into a patient, or employed to stimulate T cells to provide antigen-specific T cells which may, in turn, be administered to a patient. The use of peptide-pulsed dendritic cells to generate antigen-specific T cells and the subsequent use of such antigen-specific T cells to eradicate disease in a murine model has been demonstrated by Cheever et al, Immunological Reviews, 157:177, 1997). Additionally, vectors expressing the disclosed polynucleotides may be introduced into stem cells taken from the patient and clonally propagated in vitro for autologous transplant back into the same patient.

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Within certain aspects, polypeptides, polynucleotides, T cells and/or binding agents disclosed herein may be incorporated into pharmaceutical compositions or immunogenic compositions (i.e., vaccines). Alternatively, a pharmaceutical composition may comprise an antigen-presenting cell (e.g. a dendritic cell) transfected with a Chlamydial polynucleotide such that the antigen presenting cell expresses a Chlamydial polypeptide. Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more such compounds and an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (e.g., polylactic galactide) and liposomes (into which the compound is incorporated; see e.g., Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic

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portions of other *Chlamydial* antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

A pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated in situ. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, Crit. Rev. Therap. Drug Carrier Systems 15:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope.

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In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, adenovirus, baculovirus, togavirus, bacteriophage, and the like), which often involves the use of a non-pathogenic (defective), replication competent virus.

For example, many viral expression vectors are derived from viruses of the retroviridae family. This family includes the murine leukemia viruses, the mouse mammary tumor viruses, the human foamy viruses, Rous sarcoma virus, and the immunodeficiency viruses, including human, simian, and feline. Considerations when designing retroviral expression vectors are discussed in Comstock *et al.* (1997).

Excellent murine leukemia virus (MLV)-based viral expression vectors have been developed by Kim et al. (1998). In creating the MLV vectors, Kim et al. found that the entire gag sequence, together with the immediate upstream region, could be deleted without significantly affecting viral packaging or gene expression. Further, it was found that nearly the entire U3 region could be replaced with the immediately-early promoter of human cytomegalovirus without deleterious effects. Additionally, MCR and internal ribosome entry sites (IRES) could be added without adverse effects. Based on their observations, Kim et al. have designed a series of MLV-based expression vectors comprising one or more of the features described above.

As more has been learned about human foamy virus (HFV), characteristics of HFV that are favorable for its use as an expression vector have been discovered. These characteristics include the expression of pol by splicing and start of

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translation at a defined initiation codon. Other aspects of HFV viral expression vectors are reviewed in Bodem et al. (1997).

Murakami et al. (1997) describe a Rous sarcoma virus (RSV)-based replication-competent avian retrovirus vectors, IR1 and IR2 to express a heterologous gene at a high level. In these vectors, the IRES derived from encephalomyocarditis virus (EMCV) was inserted between the env gene and the heterologous gene. The IR1 vector retains the splice-acceptor site that is present downstream of the env gene while the IR2 vector lacks it. Murakami et al. have shown high level expression of several different heterologous genes by these vectors.

Recently, a number of lentivirus-based retroviral expression vectors have been developed. Kafri et al. (1997) have shown sustained expression of genes delivered directly into liver and muscle by a human immunodeficiency virus (HIV)-based expression vector. One benefit of the system is the inherent ability of HIV to transduce non-dividing cells. Because the viruses of Kafri et al. are pseudotyped with vesicular stomatitis virus G glycoprotein (VSVG), they can transduce a broad range of tissues and cell types.

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A large number of adenovirus-based expression vectors have been developed, primarily due to the advantages offered by these vectors in gene therapy applications. Adenovirus expression vectors and methods of using such vectors are the subject of a number of United States patents, including United States Patent No. 5,698,202, United States Patent No. 5,616,326, United States Patent No. 5,585,362, and United States Patent No. 5,518,913, all incorporated herein by reference.

Additional adenoviral constructs are described in Khatri et al. (1997) and Tomanin et al. (1997). Khatri et al. describe novel ovine adenovirus expression vectors and their ability to infect bovine nasal turbinate and rabbit kidney cells as well as a range of human cell type, including lung and foreskin fibroblasts as well as liver, prostate, breast, colon and retinal lines. Tomanin et al. describe adenoviral expression vectors containing the T7 RNA polymerase gene. When introduced into cells containing a heterologous gene operably linked to a T7 promoter, the vectors were able to drive gene expression from the T7 promoter. The authors suggest that this system may be useful for the cloning and expression of genes encoding cytotoxic proteins.

Poxviruses are widely used for the expression of heterologous genes in mammalian cells. Over the years, the vectors have been improved to allow high expression of the heterologous gene and simplify the integration of multiple heterologous genes into a single molecule. In an effort to diminish cytopathic effects and to increase safety, vaccinia virus mutant and other poxviruses that undergo abortive

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infection in mammalian cells are receiving special attention (Oertli et al., 1997). The use of poxviruses as expression vectors is reviewed in Carroll and Moss (1997).

Togaviral expression vectors, which includes alphaviral expression vectors have been used to study the structure and function of proteins and for protein production purposes. Attractive features of togaviral expression vectors are rapid and efficient gene expression, wide host range, and RNA genomes (Huang, 1996). Also, recombinant vaccines based on alphaviral expression vectors have been shown to induce a strong humoral and cellular immune response with good immunological memory and protective effects (Tubulekas *et al.*, 1997). Alphaviral expression vectors and their use are discussed, for example, in Lundstrom (1997).

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In one study, Li and Garoff (1996) used Semliki Forest virus (SFV) expression vectors to express retroviral genes and to produce retroviral particles in BHK-21 cells. The particles produced by this method had protease and reverse transcriptase activity and were infectious. Furthermore, no helper virus could be detected in the virus stocks. Therefore, this system has features that are attractive for its use in gene therapy protocols.

Baculoviral expression vectors have traditionally been used to express heterologous proteins in insect cells. Examples of proteins include mammalian chemokine receptors (Wang et al., 1997), reporter proteins such as green fluorescent protein (Wu et al., 1997), and FLAG fusion proteins (Wu et al., 1997; Koh et al., 1997). Recent advances in baculoviral expression vector technology, including their use in virion display vectors and expression in mammalian cells is reviewed by Possee (1997). Other reviews on baculoviral expression vectors include Jones and Morikawa (1996) and O'Reilly (1997).

Other suitable viral expression systems are disclosed, for example, in Fisher-Hoch et al., *Proc. Natl. Acad. Sci. USA* 86:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci.* 569:86-103, 1989; Flexner et al., *Vaccine* 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques* 6:616-627, 1988; Rosenfeld et al., *Science* 252:431-434, 1991; Kolls et al., *Proc. Natl. Acad. Sci. USA* 91:215-219, 1994; Kass-Eisler et al., *Proc. Natl. Acad. Sci. USA* 90:11498-11502, 1993; Guzman et al., *Circulation* 88:2838-2848, 1993; and Guzman et al., *Cir. Res.* 73:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. In other systems, the DNA may be introduced as "naked" DNA, as described, for example, in Ulmer et al., *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The

uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

It will be apparent that a vaccine may comprise a polynucleotide and/or a polypeptide component, as desired. It will also be apparent that a vaccine may contain pharmaceutically acceptable salts of the polynucleotides and/or polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (e.g., salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (e.g., sodium, potassium, lithium, ammonium, calcium and magnesium salts). While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Such compositions may also comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

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Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, Bortadella pertussis or Mycobacterium tuberculosis derived proteins. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant

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and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, under select circumstances, the adjuvant composition may be designed to induce an immune response predominantly of the Th1 type or Th2 type. High levels of Th1-type cytokines (e.g., IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, Ann. Rev. Immunol. 7:145-173, 1989.

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Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; see US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Thl response. Such oligonucleotides are well known and are described, for example, in WO 96/02555 and WO 99/33488. Immunostimulatory DNA sequences are also described, for example, by Sato et al., Science 273:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

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Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (e.g., SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Corixa Corporation; Seattle, WA), RC-529 (Corixa Corporation; Seattle, WA) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties.

Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immunostimulant and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (i.e., a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (see, e.g., Coombes et al., Vaccine 14:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

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Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-coglycolide), as well as polyacrylate, latex, starch, cellulose and dextran. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (e.g., a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (see e.g., U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets *Chlamydia*-infected cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the

antigen, to improve activation and/or maintenance of the T cell response, to have anti-Chlamydia effects per se and/or to be immunologically compatible with the receiver (i.e., matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

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Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, Nature 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic immunity (see Timmerman and Levy, Ann. Rev. Med. 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate in situ, with marked cytoplasmic processes (dendrites) visible in vitro), their ability to take up, process and present antigens with high efficiency, and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells in vivo or ex vivo, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (see Zitvogel et al., Nature Med. 4:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcy receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and

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class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a Chlamydial protein (or portion or other variant thereof) such that the Chlamydial polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place ex vivo, and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs in vivo. In vivo and ex vivo transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., Immunology and cell Biology 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the Chlamydial polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

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Routes and frequency of administration of pharmaceutical compositions and vaccines, as well as dosage, will vary from individual to individual. In general, the pharmaceutical compositions and vaccines may be administered by injection (e.g., intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (e.g., by aspiration) or orally. Between 1 and 3 doses may be administered for a 1-36 week period. Preferably, 3 doses are administered, at intervals of 3-4 months, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from *Chlamydial* infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 µg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

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In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a *Chlamydial* protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

In another aspect, the present invention provides methods for using the polypeptides described above to diagnose Chlamydial infection. In this aspect, methods are provided for detecting Chlamydial infection in a biological sample, using one or more of the above polypeptides, either alone or in combination. For clarity, the term "polypeptide" will be used when describing specific embodiments of the inventive diagnostic methods. However, it will be clear to one of skill in the art that the fusion proteins of the present invention may also be employed in such methods.

As used herein, a "biological sample" is any antibody-containing sample obtained from a patient. Preferably, the sample is whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood, serum or plasma sample obtained from a patient. The polypeptides are used in an assay, as described below, to determine the presence or absence of antibodies to the polypeptide(s) in the sample, relative to a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to *Chlamydia* antigens which may be indicative of *Chlamydia*-infection.

In embodiments in which more than one polypeptide is employed, the polypeptides used are preferably complementary (i.e., one component polypeptide will tend to detect infection in samples where the infection would not be detected by another

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component polypeptide). Complementary polypeptides may generally be identified by using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with *Chlamydia*. After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be formulated that are capable of detecting infection in most, or all, of the samples tested.

A variety of assay formats are known to those of ordinary skill in the art for using one or more polypeptides to detect antibodies in a sample. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988, which is incorporated herein by reference. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that contains a reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled with a reporter group (e.g., in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate, or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

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The polypeptides may be bound to the solid support using a variety of techniques known to those of ordinary skill in the art. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general,

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contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1  $\mu$ g, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (see, e.g., Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

More specifically, once the polypeptide is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin (BSA) or Tween 20<sup>TM</sup> (Sigma Chemical Co., St. Louis, MO) may be employed. The immobilized polypeptide is then incubated with the sample, and antibody is allowed to bind to the antigen. The sample may be diluted with a suitable dilutent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (i.e., incubation time) is that period of time that is sufficient to detect the presence of antibody within an HGE-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20<sup>TM</sup>. Detection reagent may then be added to the solid support. An appropriate detection reagent is any

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compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many commercial sources (e.g., Zymed Laboratories, San Francisco, CA, and Pierce, Rockford, IL).

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The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-Chlamydia antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for Chlamydia-infection. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., Clinical Epidemiology: A Basic Science for Clinical Medicine, Little Brown and Co., 1985, pp. 106-107. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (i.e., sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off

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value on the plot that is the closest to the upper left-hand corner (i.e., the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for Chlamydial infection.

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In a related embodiment, the assay is performed in a rapid flow-through or strip test format, wherein the antigen is immobilized on a membrane, such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (e.g., protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-Chlamydia antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 µg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (e.g., one drop) of patient serum or blood.

Of course, numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to be exemplary only. One example of an alternative assay protocol which may be usefully employed in such methods is a Western blot, wherein the proteins present in a biological sample are separated on a gel, prior to exposure to a binding agent. Such techniques are well known to those of skill in the art.

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a *Chlamydial* protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically

bind" to a *Chlamydial* protein if it reacts at a detectable level (within, for example, an ELISA) with a *Chlamydial* protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10<sup>3</sup> L/mol. The binding constant may be determined using methods well known in the art.

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Binding agents may be further capable of differentiating between patients with and without a *Chlamydial* infection using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a *Chlamydial* protein will generate a signal indicating the presence of a *Chlamydial* infection in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without infection. To determine whether a binding agent satisfies this requirement, biological samples (e.g., blood, sera, sputum urine and/or tissue biopsies) from patients with and without *Chlamydial* infection (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen

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without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, Eur. J. Immunol. 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

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Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane,

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Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include <sup>90</sup>Y, <sup>123</sup>I, <sup>125</sup>I, <sup>131</sup>I, <sup>186</sup>Re, <sup>188</sup>Re, <sup>211</sup>At, and <sup>212</sup>Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

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A therapeutic agent may be coupled (e.g., covalently bonded) to a suitable monoclonal antibody either directly or indirectly (e.g., via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, e.g., U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction

of a disulfide bond (e.g., U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (e.g., U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (e.g., U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (e.g., U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (e.g., U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers which provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

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A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (e.g., U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (e.g., U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (e.g., U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in site-specific regions by appropriate methods. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density, and the rate of clearance of the antibody.

Antibodies may be used in diagnostic tests to detect the presence of *Chlamydia* antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting Chlamydial infection in a patient.

Diagnostic reagents of the present invention may also comprise DNA sequences encoding one or more of the above polypeptides, or one or more portions

thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify *Chlamydia*-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule encoding a polypeptide of the present invention. The presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a DNA molecule encoding a polypeptide of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

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As used herein, the term "oligonucleotide primer/probe specific for a DNA molecule" means an oligonucleotide sequence that has at least about 80%, preferably at least about 90% and more preferably at least about 95%, identity to the DNA molecule in question. Oligonucleotide primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 10-40 nucleotides. In a preferred embodiment, the oligonucleotide primers comprise at least about 10 contiguous nucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (see, for example, Mullis et al. Ibid; Ehrlich, Ibid). Primers or probes may thus be used to detect Chlamydia-specific sequences in biological samples. DNA probes or primers comprising oligonucleotide sequences described above may be used alone or in combination with each other.

The following Examples are offered by way of illustration and not by way of limitation.

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## EXAMPLE 1

## ISOLATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS

Chlamydia antigens of the present invention were isolated by expression cloning of a genomic DNA library of Chlamydia trachomatis LGV II essentially as described by Sanderson et al. (J. Exp. Med., 1995, 182:1751-1757) and were shown to induce PBMC proliferation and IFN-γ in an immunoreactive T cell line.

A Chlamydia-specific T cell line was generated by stimulating PBMCs from a normal donor with no history of chlamydial genital tract infection with elementary bodies of Chlamydia trachomatis LGV II. This T cell line, referred to as TCL-8, was found to recognize both Chlamydia trachomatis and Chlamydia pneumonia infected monocyte-derived dendritic cells.

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A randomly sheared genomic library of Chlamydia trachomatis LGV II was constructed in Lambda ZAP (Stratagene, La Jolla, CA) and the amplified library plated out in 96 well microtiter plates at a density of 30 clones/well. Bacteria were induced to express recombinant protein in the presence of 2 mM IPTG for 3 h, then pelleted and resuspended in 200 μl of RPMI 10% FBS. 10 μl of the induced bacterial suspension was transferred to 96 well plates containing autologous monocyte-derived dendritic cells. After a 2 h incubation, dendritic cells were washed to remove free E. coli and Chlamydia-specific T cells were added. Positive E. coli pools were identified by determining IFN-γ production and proliferation of the T cells in response to the pools.

Four positive pools were identified, which were broken down to yield four pure clones (referred to as 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31), with insert sizes of 481 bp, 183 bp, 110 bp and 1400 bp, respectively. The determined DNA sequences for 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31 are provided in SEQ ID NO: 1-4, respectively. Clone 1-B1-66 is approximately in region 536690 of the *C. trachomatis* genome (NCBI *C. trachomatis* database). Within clone 1-B1-66, an open reading frame (ORF) has been identified (nucleotides 115 - 375) that encodes a previously identified 9 kDa protein (Stephens, et al. Genbank Accession No. AE001320), the sequence of which is provided in SEQ ID NO: 5). Clone 4-D7-28 is a smaller region of the same ORF (amino acids 22-82 of 1-B1-66). Clone 3-G3-10 is approximately in region 74559 of the *C. trachomatis* genome. The insert is cloned in the antisense orientation with respect to its orientation in the genome. The clone 10-C10-31 contains an open reading frame that corresponds to a previously published sequence for S13 ribosomal protein from *Chlamydia trachomatis* (Gu, L. et al. *J. Bacteriology*, 177:2594-2601, 1995). The predicted protein sequences for 4-D7-28 and

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10-C10-31 are provided in SEQ ID NO: 6 and 12, respectively. Predicted protein sequences for 3-G3-10 are provided in SEQ ID NO: 7-11.

In a related series of screening studies, an additional T cell line was used to screen the genomic DNA library of *Chlamydia trachomatis* LGV II described above. A *Chlamydia*-specific T cell line (TCT-1) was derived from a patient with a chlamydial genital tract infection by stimulating patient PBMC with autologous monocyte-derived dendritic cells infected with elementary bodies of *Chlamydia trachomatis* LGV II. One clone, 4C9-18 (SEQ ID NO: 21), containing a 1256 bp insert, elicited a specific immune response, as measured by standard proliferation assays, from the *Chlamydia*-specific T cell line TCT-1. Subsequent analysis revealed this clone to contain three known sequences: lipoamide dehydrogenase (Genbank Accession No. AE001326), disclosed in SEQ ID NO: 22; a hypothetical protein CT429 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 23; and part of an open reading frame of ubiquinone methyltransferase CT428 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 24.

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In further studies involving clone 4C9-18 (SEQ ID NO: 21), the full-length amino acid sequence for lipoamide dehydrognase (SEQ ID NO: 22) from *C. trachomatis* (LGV II) was expressed in clone CtL2-LPDA-FL, as disclosed in SEQ ID NO: 90.

To further characterize the open reading frame containing the T cell stimulating epitope(s), a cDNA fragment containing nucleotides 1-695 of clone 4C9-18 with a cDNA sequence encoding a 6X-Histidine tag on the amino terminus was subcloned into the Ndel/EcoRI site of the pET17b vector (Novagen, Madison, WI), referred to as clone 4C9-18#2 BL21 pLysS (SEQ ID NO: 25, with the corresponding amino acid sequence provided in SEQ ID NO: 26) and transformed into E. coli. Selective induction of the transformed E. coli with 2 mM IPTG for three hours resulted in the expression of a 26 kDa protein from clone 4C9-18#2 BL21 pLysS, as evidenced by standard Coomassie-stained SDS-PAGE. To determine the immunogenicity of the protein encoded by clone 4C9-18#2 BL21 pLysS, E. coli expressing the 26 kDa protein were titered onto 1 x 10<sup>4</sup> monocyte-derived dendritic cells and incubated for two hours. The dendritic cell cultures were washed and 2.5 x 10<sup>4</sup> T cells (TCT-1) added and allowed to incubate for an additional 72 hours, at which time the level of IFN-y in the culture supernatant was determined by ELISA. As shown in Fig. 1, the T-cell line TCT-1 was found to respond to induced cultures as measured by IFN-g, indicating a Chlamydia-specific T-cell response against the lipoamide dehydrogenase sequence.

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Similarly, the protein encoded by clone 4C9-18#2 BL21 pLysS was shown to stimulate the TCT-1 T-cell line by standard proliferation assays.

Subsequent studies to identify additional *Chlamydia trachomatis* antigens using the above-described CD4+ T-cell expression cloning technique yielded additional clones. The TCT-1 and TCL-8 *Chlamydia*-specific T-cell lines, as well as the TCP-21 T-cell line were utilized to screen the *Chlamydia trachomatis* LGVII genomic library. The TCP-21 T-cell line was derived from a patient having a humoral immune response to *Chlamydia pnuemoniae*. The TCT-1 cell line identified 37 positive pools, the TCT-3 cell line identified 41 positive pools and the TCP-21 cell line identified 2 positive pools. The following clones were derived from 10 of these positive pools. Clone 11-A3-93 (SEQ ID NO: 64), identified by the TCP-21 cell line, is a 1339 bp genomic fragment sharing homology to the HAD superfamily (CT103). The second insert in the same clone shares homology with the fab I gene (CT104) present on the complementary strand. Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of *C. pnuemoniae*.

Clone 11-G10-46, (SEQ ID NO: 62), identified using the TCT-3 cell line, contains a 688 bp insert that shares homology to the hypothetical protein CT610. Clone 11-G1-34, (SEQ ID NO: 61), identified using the TCT-3 cell line, has two partial open reading frames (ORF) with an insert size of 1215 bp. One ORF shares homology to the malate dehydrogenase gene (CT376), and the other ORF shares homology to the glycogen hydrolase gene (CT042). Clone 11-H3-68, (SEQ ID NO: 60), identified using the TCT-3 cell line, has two ORFs with a total insert size of 1180 bp. One partial ORF encodes the plasmid-encoded PGP6-D virulence protein while the second ORF is a complete ORF for the L1 ribosomal gene (CT318). Clone 11-H4-28, (SEQ ID NO: 59), identified using the TCT-3 cell line, has an insert size of 552 bp and is part of the ORF for the dnaK gene (CT396). Clone 12-B3-95, (SEQ ID NO: 58), identified using the TCT-1 cell line, has an insert size of 463 bp and is a part of the ORF for for the lipoamide dehydrogenase gene (CT557). Clones 15-G1-89 and 12-B3-95 are identical, (SEQ ID NO: 55 and 58, respectively), identified using the TCT-1 cell line, has an insert size of 463 bp and is part of the ORF for the lipoamide dehydrogenase gene (CT557). Clone 12-G3-83, (SEQ ID NO: 57), identified using the TCT-1 cell line, has an insert size of 1537 bp and has part of the ORF for the hypothetical protein CT622.

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Clone 23-G7-68, (SEQ ID NO: 79), identified using the TCT-3 cell line, 35 contains a 950 bp insert and contains a small part of the L11 ribosomal ORF, the entire ORF for L1 ribosomal protein and a part of the ORF for L10 ribosomal protein. Clone

22-F8-91, (SEQ ID NO: 80), identified using the TCT-1 cell line, contains a 395 bp insert that contains a part of the pmpC ORF on the complementary strand of the clone. Clone 21-E8-95, (SEQ ID NO: 81), identified using the TCT-3 cell line, contains a 2,085 bp insert which contains part of CT613 ORF, the complete ORF for CT612, the complete ORF for CT611 and part of the ORF for CT610. Clone 19-F12-57, (SEQ ID NO: 82), identified using the TCT-3 cell line, contains a 405 bp insert which contains part of the CT 858 ORF and a small part of the recA ORF. Clone 19-F12-53, (SEQ ID NO: 83), identified using the TCT-3 cell line, contains a 379 bp insert that is part of the ORF for CT455 encoding glutamyl tRNA synthetase. Clone 19-A5-54, (SEQ ID NO: 84), identified using the TCT-3 cell line, contains a 715 bp insert that is part of the ORF3 (complementary strand of the clone) of the cryptic plasmid. Clone 17-E11-72, (SEQ ID NO: 85), identified using the TCT-1 cell line, contains a 476 bp insert that is part of the ORF for Opp\_2 and pmpD. The pmpD region of this clone is covered by the pmpD region of clone 15-H2-76. Clone 17-C1-77, (SEQ ID NO: 86), identified using the TCT-3 cell line, contains a 1551 bp insert that is part of the CT857 ORF, as well as part of the CT858 ORF. Clone 15-H2-76, (SEQ ID NO: 87), identified using the TCT-1 cell line, contains a 3,031 bp insert that contains a large part of the pmpD ORF, part of the CT089 ORF, as well as part of the ORF for SycE. Clone 15-A3-26, (SEQ ID NO: 88), contains a 976 bp insert that contains part of the ORF for CT858. Clone 17-G4-36, (SEQ ID NO: 267), identified using the TCT-10 cell line, contains a 680 bp insert that 20 is in frame with beta-gal in the plasmid and shares homology to part of the ORF for DNA-directed RNA polymerase beta subunit (CT315 in SerD).

Several of the clones described above share homology to various polymorphic membrane proteins. The genomic sequence of *Chlamydia trachomatis* contains a family of nine polymorphic membrane protein genes, referred to as pmp. These genes are designated pmpA, pmpB, pmpC, pmpD, pmpE, pmpF, pmpG, pmpH and pmpI. Proteins expressed from these genes are believed to be of biological relevance in generating a protective immune response to a *Chlamydial* infection. In particular, pmpC, pmpD, pmpE and pmpI contain predictable signal peptides, suggesting they are outer membrane proteins, and therefore, potential immunological targets.

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Based on the Chlamydia trachomatis LGVII serovar sequence, primer pairs were designed to PCR amplify the full-length fragments of pmpC, pmpD, pmpE, pmpG, pmpH and pmpI. The resulting fragments were subcloned into the DNA vaccine vector JA4304 or JAL, which is JA4304 with a modified linker (SmithKline Beecham, London, England). Specifically, PmpC was subcloned into the JAL vector using the 5'

oligo GAT AGG CGC GCC GCA ATC ATG AAA TTT ATG TCA GCT ACT GCT G and the 3' oligo CAG AAC GCG TTT AGA ATG TCA TAC GAG CAC CGC A, as provided in SEQ ID NO: 197 and 198, respectively. PCR amplification of the gene under conditions well known in the art and ligation into the 5' ASCI/3' MluI sites of the JAL vector was completed after inserting the short nucleotide sequence GCAATC (SEQ ID NO: 199) upstream of the ATG to create a Kozak-like sequence. The resulting expression vector contained the full-length pmpC gene comprising 5325 nucleotides (SEQ ID NO: 173) containing the hypothetical signal sequence, which encodes a 187 kD protein (SEQ ID NO: 179). The pmpD gene was subcloned into the JA4304 vaccine vector following PCR amplification of the gene using the following oligos: 5' oligo-TGC AAT CAT GAG TTC GCA GAA AGA TAT AAA AAG C (SEQ ID NO: 200) and 3' oligo- CAG AGC TAG CTT AAA AGA TCA ATC GCA ATC CAG TAT TC (SEQ ID NO: 201). The gene was ligated into the a 5' blunted HIII/3' MluI site of the JA4304 vaccine vector using standard techniques well known in the art. The CAATC (SEQ ID NO: 202) was inserted upstream of the ATG to create a Kozak-like sequence. This clone is unique in that the last threonine of the HindIII site is missing due to the blunting procedure, as is the last glycine of the Kozak-like sequence. The insert, a 4593 nucleotide fragment (SEQ ID NO: 172) is the full-length gene for pmpD containing the hypothetical signal sequence, which encodes a 161 kD protein (SEQ ID NO: 178). PmpE was subcloned into the JA4304 vector using the 5' oligo- TGC AAT CAT GAA AAA AGC GTT TTT CTT TTT C (SEQ ID NO: 203), and the 3' oligo- CAG AAC GCG TCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 204). Following PCR amplification, the gene was ligated into the 5' blunted HIII/3' MluI site of JA4304. To facilitate this, a short nucleotide sequence, TGCAATC (SEQ ID NO: 293), was added upstream of the initiation codon for creating a Kozak-like sequence and reconstituting 25 the HindIII site. The insert is the full-length pmpE gene (SEQ ID NO: 171) containing the hypothetical signal sequence. The pmpE gene encodes a 105 kD protein (SEQ ID NO: 177). The pmpG gene was PCR amplified using the 5' oligo- GTG CAA TCA TGA TTC CTC AAG GAA TTT ACG ( SEQ ID NO: 205), and the 3' oligo- CAG AAC GCG TTT AGA ACC GGA CTT TAC TTC C (SEQ ID NO: 206) and subcloned into the JA4304 vector. Similar cloning strategies were followed for the pmpI and pmpK genes. In addition, primer pairs were designed to PCR amplify the full-length or overlapping fragments of the pmp genes, which were then subcloned for protein expression in the pET17b vector (Novagen, Madison, WI) and transfected into E. coli BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel chromatographic methodology provided by Novagen. Several of the genes encoding

the recombinant proteins, as described below, lack the native signal sequence to facilitate expression of the protein. Full-length protein expression of pmpC was accomplished through expression of two overlapping fragments, representing the amino and carboxy termini. Subcloning of the pmpC-amino terminal portion, which lacks the signal sequence, (SEQ ID NO: 187, with the corresponding amino acid sequence provided in SEQ ID NO: 195) used the 5' oligo- CAG ACA TAT GCA TCA CCA TCA CCA TCA CGA GGC GAG CTC GAT CCA AGA TC (SEQ ID NO: 207), and the 3' oligo- CAG AGG TAC CTC AGA TAG CAC TCT CTC CTA TTA AAG TAG G (SEQ ID NO: 208) into the 5' NdeI/3' KPN cloning site of the vector. The carboxy terminus portion of the gene, pmpC-carboxy terminal fragment (SEQ ID NO: 186, with the corresponding amino acid sequence provided in SEQ ID NO: 194), was subcloned into the 5' NheI/3' KPN cloning site of the expression vector using the following primers: 5' oligo- CAG AGC TAG CAT GCA TCA CCA TCA CCA TCA CGT TAA GAT TGA GAA CTT CTC TGG C (SEQ ID NO: 209), and 3' oligo- CAG AGG TAC CTT AGA ATG TCA TAC GAG CAC CGC AG (SEQ ID NO: 210). PmpD was also expressed as two overlapping proteins. The pmpD-amino terminal portion, which lacks the signal sequence, (SEQ ID NO: 185, with the corresponding amino acid sequence provided in SEQ ID NO: 193) contains the initiating codon of the pET17b and is expressed as a 80 kD protein. For protein expression and purification purposes, a sixhistidine tag follows the initiation codon and is fused at the 28th amino acid (nucleotide 20 84) of the gene. The following primers were used, 5' oligo, CAG ACA TAT GCA TCA CCA TCA CCA TCA CGG GTT AGC (SEQ ID NO: 211), and the 3' oligo- CAG AGG TAC CTC AGC TCC TCC AGC ACA CTC TCT TC (SEQ ID NO: 212), to splice into the 5' NdeI/3' KPN cloning site of the vector. The pmpD-carboxy terminus portion (SEQ ID NO: 184) was expressed as a 92 kD protein (SEQ ID NO: 192). For 25 expression and subsequent purification, an additional methionine, alanine and serine was included, which represent the initiation codon and the first two amino acids from the pET17b vector. A six-histidine tag downstream of the methionine, alanine and serine is fused at the 691st amino acid (nucleotide 2073) of the gene. The 5' oligo- CAG AGC TAG CCA TCA CCA TCA CGG TGC TAT TTC TTG CTT ACG TGG (SEQ ID NO: 213) and the 3' oligo- CAG AGG TAC TTn AAA AGA TCA ATC GCA ATC CAG TAT TCG (SEQ ID NO: 214) were used to subclone the insert into the 5' NheI/3' KPN cloning site of the expression vector. PmpE was expressed as a 106kD protein (SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191). The pmpE insert also lacks the native signal sequence. amplification of the gene under conditions well known in the art was performed using 10

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the following oligo primers: 5' oligo- CAG AGG ATC CAC ATC ACC ATC ACC ATC ACG GAC TAG CTA GAG AGG TTC (SEQ ID NO: 215), and the 3' oligo-CAG AGA ATT CCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 216), and the amplified insert was ligated into a 5' BamHI/3' EcoRI site of JA4304. The short nucleotide sequence, as provided in SEQ ID NO: 217, was inserted upstream of the initiation codon for creating the Kozak-like sequence and reconstituting the HindIII site. The expressed protein contains the initiation codon and the downstream 21 amino acids from the pET17b expression vector, i.e., MASMTGGQQMGRDSSLVPSSDP (SEQ ID NO: 218). In addition, a six-histidine tag is included upstream of the sequence described above and is fused at the 28th amino acid (nucleotide 84) of the gene, which eliminates the hypothetical signal peptide. The sequences provided in SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191 do not include these additional sequences. The pmpG gene (SEQ ID NO: 182, with the corresponding amino acid sequence provided in SEQ ID No; 190) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo-CAG AGG TAC CGC ATC ACC ATC ACC ATC ACA TGA TTC CTC AAG GAA TTT ACG (SEQ ID NO: 219), and the 3' oligo- CAG AGC GGC CGC TTA GAA CCG GAC TTT ACT TCC (SEQ ID NO: 220), and ligated into the 5' KPN/3' NotI cloning site of the expression vector. The expressed protein contains an additional namely, amino end, at the sequence amino acid MASMTGGQQNGRDSSLVPHHHHHHH (SEQ ID NO: 221), which comprises the initiation codon and additional sequence from the pET17b expression vector. The pmpI gene (SEQ ID NO: 181, with the corresponding amino acid sequence provided in SEQ ID No; 189) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo- CAG AGC TAG CCA TCA CCA TCA CCA TCA CCT CTT TGG CCA GGA TCC C (SEQ ID NO: 222), and the 3' oligo- CAG AAC TAG TCT AGA ACC TGT AAG TGG TCC (SEQ ID NO: 223), and ligted into the expression vector at the 5' NheI/3' SpeI cloning site. The 95 kD expressed protein contains the initiation codon plus an additional alanine and serine from the pET17b vector at the amino end of the protein. In addition, a six-histidine tag is fused at the 21st amino acid of the gene, which eliminates the hypothetical signal peptide.

Clone 14H1-4, (SEQ ID NO: 56), identified using the TCT-3 cell line, contains a complete ORF for the TSA gene, thiol specific antioxidant — CT603 (the CT603 ORF is a homolog of CPn0778 from *C. pnuemoniae*). The TSA open reading frame in clone 14-H1-4 was amplified such that the expressed protein possess an additional methionine and a 6x histidine tag (amino terminal end). This amplified insert

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was sub-cloned into the Nde/EcoRI sites of the pET17b vector. Upon induction of this clone with IPTG, a 22.6 kDa protein was purified by Ni-NTA agarose affinity chromatography. The determined amino acid sequence for the 195 amino acid ORF of clone 14-H1-4 encoding the TSA gene is provided in SEQ ID NO: 65. Further analysis yielded a full-length clone for the TSA gene, referred to as CTL2-TSA-FL, with the full-length amino acid sequence provided in SEQ ID NO: 92.

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Further studies yielded 10 additional clones identified by the TCT-1 and TCT-3 T-cell lines, as described above. The clones identified by the TCT-1 line are: 16-D4-22, 17-C5-19, 18-C5-2, 20-G3-45 and 21-C7-66; clones identified by the TCT-3 cell line are: 17-C10-31, 17-E2-9, 22-A1-49 and 22-B3-53. Clone 21-G12-60 was recognized by both the TCT-1 and TCT-3 T cell lines. Clone 16-D4-22 (SEQ ID NO: 119), identified using the TCT-1 cell line contains a 953 bp insert that contains two genes, parts of open reading frame 3 (ORF3) and ORF4 of the C. trachomatis plasmid for growth within mammalian cells. Clone 17-C5-19 (SEQ ID NO: 118), contains a 951 bp insert that contains part of the ORF for DT431, encoding for clpP\_1 protease and part of the ORF for CT430 (diaminopimelate epimerase). Clone 18-C5-2 (SEQ ID NO: 117) is part of the ORF for S1 ribosomal protein with a 446 bp insert that was identified using the TCT-1 cell line. Clone 20-G3-45 (SEQ ID NO: 116), identified by the TCT-1 cell line, contains a 437 bp insert that is part of the pmpB gene (CT413). Clone 21-C7-66 (SEQ ID NO: 115), identified by the TCT-1 line, contains a 995bp insert that encodes part of the dnaK like protein. The insert of this clone does not overlap with the insert of the TCT-3 clone 11-H4-28 (SEQ ID NO: 59), which was Clone 17-C10-31 (SEQ ID NO: 114), shown to be part of the dnaK gene CT396 identified by the TCT-3 cell line, contains a 976 bp insert. This clone contains part of the ORF for CT858, a protease containing IRBP and DHR domains. Clone 17-E2-9 25 (SEQ ID NO: 113) contains part of ORFs for two genes, CT611 and CT610, that span a 1142 bp insert. Clone 22-A1-49 (SEQ ID NO: 112), identified using the TCT-3 line, also contains two genes in a 698 bp insert. Part of the ORF for CT660 (DNA gyrase(gyrA 2)) is present on the top strand where as the complete ORF for a hypothetical protein CT659 is present on the complementary strand. Clone 22-B3-53 (SEQ ID NO: 111), identified by the TCT-1 line, has a 267 bp insert that encodes part of the ORF for GroEL (CT110). Clone 21-G12-60 (SEQ ID NO: 110), identified by both the TCT-1 and TCT-3 cell lines contains a 1461 bp insert that contains partial ORFs for hypothetical proteins CT875, CT229 and CT228.

Additional Chlamydia antigens were obtained by screening a genomic expression library of Chlamydia trachomatis (LGV II serovar) in Lambda Screen-1

vector (Novagen, Madison, WI) with sera pooled from several Chlamydia-infected individuals using techniques well known in the art. The following immuno-reactive clones were identified and the inserts containing Chlamydia genes sequenced: CTL2#1 (SEQ ID NO: 71); CTL2#2 (SEQ ID NO: 70); CTL2#3-5' (SEQ ID NO: 72, a first determined genomic sequence representing the 5' end); CTL2#3-3' (SEQ ID NO: 73, a second determined genomic sequence representing the 3' end); CTL2#4 (SEQ ID NO: 53); CTL2#5 (SEQ ID NO: 69); CTL2#6 (SEQ ID NO: 68); CTL2#7 (SEQ ID NO: 67); CTL2#8b (SEQ ID NO: 54); CTL2#9 (SEQ ID NO: 66); CTL2#10-5' (SEQ ID NO: 74, a first determined genomic sequence representing the 5' end); CTL2#10-3' (SEQ ID NO: 75, a second determined genomic sequence representing the 3' end); CTL2#11-5' (SEQ ID NO: 45, a first determined genomic sequence representing the 5' end); CTL2#11-3' (SEQ ID NO: 44, a second determined genomic sequence representing the 3' end); CTL2#12 (SEQ ID NO: 46); CTL2#16-5' (SEQ ID NO: 47); CTL2#18-5' (SEQ ID NO: 49, a first determined genomic sequence representing the 5' end); 15 CTL2#18-3' (SEQ ID NO: 48, a second determined genomic sequence representing the 3' end); CTL2#19-5' (SEQ ID NO: 76, the determined genomic sequence representing the 5' end); CTL2#21 (SEQ ID NO: 50); CTL2#23 (SEQ ID NO: 51; and CTL2#24 (SEQ ID NO: 52).

Additional Chlamydia trachomatis antigens were identified by These studies used sera pooled from several serological expression cloning. Chlamydia-infected individuals, as described above, but, IgA, and IgM antibodies were used in addition to IgG as a secondary antibody. Clones screened by this method enhance detection of antigens recognized by an early immune response to a Chlamydial infection, that is a mucosal humoral immune response. The following immunoreactive clones were characterized and the inserts containing Chlamydia genes sequenced: CTL2gam-1 (SEQ ID NO: 290), CTL2gam-2 (SEQ ID NO: 289), CTL2gam-5 (SEQ ID NO: 288), CTL2gam-6-3' (SEQ ID NO: 287, a second determined genomic sequence representing the 3' end), CTL2gam-6-5' (SEQ ID NO: 286, a first determined genomic sequence representing the 5' end), CTL2gam-8 (SEQ ID NO: 285), CTL2gam-10 (SEQ ID NO: 284), CTL2gam-13 (SEQ ID NO: 283), CTL2gam-15-3' (SEQ ID NO: 282, a second determined genomic sequence representing the 3' end), CTL2gam-15-5' (SEQ ID NO: 281, a first determined genomic sequence representing the 5' end), CTL2gam-17 (SEQ ID NO: 280), CTL2gam-18 (SEQ ID NO: 279), CTL2gam-21 (SEQ ID NO: 278), CTL2gam-23 (SEQ ID NO: 277), CTL2gam-24 (SEQ ID NO: 276), CTL2gam-26 (SEQ ID NO: 275), CTL2gam-27 (SEQ ID NO: 274), CTL2gam-28 (SEQ ID NO: 273), CTL2gam-30-3' (SEQ ID NO: 272, a second determined genomic sequence

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representing the 3' end) and CTL2gam-30-5' (SEQ ID NO: 271, a first determined genomic sequence representing the 5' end).

# **EXAMPLE 2**

INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y PRODUCTION BY CHLAMYDIA TRACHOMATIS ANTIGENS

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The ability of recombinant *Chlamydia trachomatis* antigens to induce T cell proliferation and interferon-γ production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatograph (Webb et al., *J. Immunology 157*:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from *C. trachomatis* patients as well as from normal donors whose T-cells are known to proliferate in response to *Chlamydia* antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 µg/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 µg/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 µl, 50 µl of medium is removed from each well for determination of IFN-γ levels, as described below. The plates are then pulsed with 1 µCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ is measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving

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an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

Using the above methodology, recombinant 1B1-66 protein (SEQ ID NO: 5) as well as two synthetic peptides corresponding to amino acid residues 48-67 5 (SEQ ID NO: 13; referred to as 1-B1-66/48-67) and 58-77 (SEQ ID NO: 14, referred to as 1B1-66/58-77), respectively, of SEQ ID NO: 5, were found to induce a proliferative response and IFN-y production in a Chlamydia-specific T cell line used to screen a genomic library of C. trachomatis LGV II.

Further studies have identified a C. trachomatis-specific T-cell epitope in the ribosomal S13 protein. Employing standard epitope mapping techniques well known in the art, two T-cell epitopes in the ribosomal S13 protein (rS13) were identified with a Chlamydia-specific T-cell line from donor CL-8 (T-cell line TCL-8 EB/DC). Fig. 8 illustrates that the first peptide, rS13 1-20 (SEQ ID NO: 106), is 100% identical with the corresponding C. pneumoniae sequence, explaining the crossreactivity of the T-cell line to recombinant C. trachomatis- and C. pneumoniae-rS13. The response to the second peptide rS13 56-75 (SEQ ID NO: 108) is C. trachomatisspecific, indicating that the rS13 response in this healthy asymptomatic donor was elicited by exposure to C. trachomatis and not to C. pneumoniae, or any other microbial infection.

As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 10<sup>4</sup> TCP-21 T-cells in the presence of 1 x 10<sup>4</sup> monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes 30 CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-21 T-cell line also gave a proliferative response to the homologous C. pneumoniae peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the C. trachomatis peptides. The amino acid substitutions in position two 35 (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative

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response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between C. trachomatis and C. pneumoniae.

To further define the epitope described above, an additional T-cell line, TCT-3, was used in epitope mapping experiments. The immunoassays were performed as described above, except that only peptides from *C. trachomatis* were tested. The T-cells gave a proliferative response to two peptides, CT-OMCB #152-171 and CT-OMCB #157-176 (SEQ ID NO: 246 and 247, respectively), thereby defining an additional immunogenic epitope in the cysteine rich outer membrane protein of *C. trachomatis*.

Clone 14H1-4, (SEQ ID NO: 56, with the corresponding full-length amino acid sequence provided in SEQ ID NO: 92), was identified using the TCT-3 cell line in the CD4 T-cell expression cloning system previously described, and was shown to contain a complete ORF for the, thiol specific antioxidant gene (CT603), referred to as TSA. Epitope mapping immunoassays were performed, as described above, to further define the epitope. The TCT-3 T-cells line exhibited a strong proliferative response to the overlapping peptides CT-TSA #96-115, CT-TSA #101-120 and CT-TSA #106-125 (SEQ ID NO: 254-256, respectively) demonstrating an immunoreactive epitope in the thiol specific antioxidant gene of *C. trachomatis* serovar LGVII.

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# **EXAMPLE 3**

# PREPARATION OF SYNTHETIC POLYPEPTIDES

Polypeptides may be synthesized on a Millipore 9050 peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugating or labeling of the peptide. Cleavage of the peptides from the solid support may be carried trifluoroacetic cleavage mixture: following out using the acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then 30 be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the Following lyophilization of the pure fractions, the peptides may be peptides. characterized using electrospray mass spectrometry and by amino acid analysis.

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### **EXAMPLE 4**

# ISOLATION AND CHARACTERIZATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS USING RETROVIRAL EXPRESSION VECTOR SYSTEMS

AND SUBSEQUENT IMMUNOLOGICAL ANALYSIS

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A genomic library of Chlamydia trachomatis LGV II was constructed by limited digests using BamHI, BglII, BstYi and MboI restriction enzymes. The restriction digest fragments were subsequently ligated into the BamHI site of the retroviral vectors pBIB-KS1,2,3. This vector set was modified to contain a Kosak translation initiation site and stop codons in order to allow expression of proteins from short DNA genomic fragments, as shown in Fig. 2. DNA pools of 80 clones were prepared and transfected into the retroviral packaging line Phoenix-Ampho, as described in Pear, W.S., Scott, M.L. and Nolan, G.P., Generation of High Titre, Helperfree Retroviruses by Transient Transfection. Methods in Molecular Medicine: Gene Therapy Protocols, Humana Press, Totowa, NJ, pp. 41-57. The Chlamydia library in retroviral form was then transduced into H2-Ld expressing P815 cells, which were then used as target cells to stimulate an antigen specific T-cell line.

A Chlamydia-specific, murine H2<sup>d</sup> restricted CD8+ T-cell line was expanded in culture by repeated rounds of stimulation with irradiated C. trachomatis-infected J774 cells and irradiated syngeneic spleen cells, as described by Starnbach, M., in J. Immunol., 153:5183, 1994. This Chlamydia-specific T-cell line was used to screen the above Chlamydia genomic library expressed by the retrovirally-transduced P815 cells. Positive DNA pools were identified by detection of IFN-γ production using Elispot analysis (see Lalvani et al., J. Experimental Medicine 186:859-865, 1997).

Two positive pools, referred to as 2C7 and 2E10, were identified by IFN-γ Elispot assays. Stable transductants of P815 cells from pool 2C7 were cloned by limiting dilution and individual clones were selected based upon their capacity to elicit IFN-γ production from the *Chlamydia*-specific CTL line. From this screening process, four positive clones were selected, referred to as 2C7-8, 2C7-9, 2C7-19 and 2C7-21. Similarly, the positive pool 2E10 was further screened, resulting in an additional positive clone, which contains three inserts. The three inserts are fragments of the CT016, tRNA syntase and clpX genes (SEQ ID NO: 268-270, respectively).

Transgenic DNA from these four positive 2C7 clones were PCR amplified using pBIB-KS specific primers to selectively amplify the *Chlamydia* DNA insert. Amplified inserts were gel purified and sequenced. One immunoreactive clone, 2C7-8 (SEQ ID NO: 15, with the predicted amino acid sequence provided in SEQ ID

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NO: 32), is a 160 bp fragment with homology to nucleotides 597304-597145 of *Chlamydia trachomatis*, serovar D (NCBI, BLASTN search; SEQ ID NO: 33, with the predicted amino acid sequence provided in SEQ ID NO: 34). The sequence of clone 2C7-8 maps within two putative open reading frames from the region of high homology described immediately above, and in particular, one of these putative open reading frames, consisting of a 298 amino acid fragment (SEQ ID NO: 16, with the predicted amino acid sequence provided in SEQ ID NO: 17), was demonstrated to exhibit immunological activity.

Full-length cloning of the 298 amino acid fragment (referred to as CT529 and/or the Capl gene) from serovar L2 was obtained by PCR amplification using 5'-ttttgaagcaggtaggtgaatatg (forward) (SEQ ID NO: 159) and 5'-ttaagaaatttaaaaaatccctta (reverse) (SEQ ID NO: 160) primers, using purified C. trachomatis L2 genomic DNA as template. This PCR product was gel-purified, cloned into pCRBlunt (Invitrogen, Carlsbad, CA) for sequencing, and then subcloned into the EcoRI site of pBIB-KMS, a derivative of pBIB-KS for expression. The Chlamydia pnuemoniae homlogue of CT529 is provided in SEQ ID NO: 291, with the corresponding amino acid sequence provided in SEQ ID NO: 292.

Full-length DNA encoding various CT529 serovars were amplified by PCR from bacterial lysates containing 10<sup>5</sup> IFU, essentially as described (Denamur, E., C. Sayada, A. Souriau, J. Orfila, A. Rodolakis and J. Elion. 1991. J. Gen. Microbiol. 137: 2525). The following serovars were amplified as described: Ba (SEQ ID NO: 134, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 135); E (BOUR) and E (MTW447) (SEQ ID NO: 122, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 123); F (NI1) (SEQ ID NO: 128, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 129); G; (SEQ ID NO: 126, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 127); Ia (SEQ ID NO: 124, with the corresponding predicted amino acid sequence provided in SEO ID NO: 125); L1 (SEQ ID NO: 130, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 131); L3 (SEQ ID NO: 132, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 133); I (SEQ ID NO: 263, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 264); K (SEQ ID NO: 265, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 266); and MoPn (SEQ ID NO: 136, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 137). PCR reactions were performed with Advantage Genomic PCR Kit (Clontech, Palo Alto, CA) using primers specific for serovar L2 DNA (external to the ORF). Primers sequences were 5'-

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ggtataatatctctctaaattttg (forward-SEQ ID NO: 161) and 5'-agataaaaaaggctgtttc' (reverse-SEQ ID NO: 162) except for MoPn which required 5'-ttttgaagcaggtaggtgaatatg (forward-SEQ ID NO: 163) and 5'-tttacaataagaaaagctaagcactttgt (reverse-SEQ ID NO: 164). PCR amplified DNA was purified with QIAquick PCR purification kit (Qiagen, Valencia, CA) and cloned in pCR2.1 (Invitrogen, Carlsbad, CA) for sequencing.

Sequencing of DNA derived from PCR amplified inserts of immunoreactive clones was done on an automated sequencer (ABI 377) using both a pBIB-KS specific forward primer 5'-cettacacagtectgetgae (SEQ ID NO: 165) and a reverse primer 3'-gttteegggeeetcacattg (SEQ ID NO: 166). PCRBlunt cloned DNA coding for CT529 serovar L2 and pCR2.1 cloned DNA coding for CT529 serovar Ba, E (BOUR), E (MTW447), F (NI1), G, Ia, K, L1, L3 and MoPn were sequenced using T7 promoter primer and universal M13 forward and M13 reverse primers.

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To determine if these two putative open reading frames (SEQ ID NO: 16 and 20) encoded a protein with an associated immunological function, overlapping peptides (17-20 amino acid lengths) spanning the lengths of the two open reading frames were synthesized, as described in Example 3. A standard chromium release assay was utilized to determine the per cent specific lysis of peptide-pulsed H2d restricted target cells. In this assay, aliquots of P815 cells (H2d) were labeled at 37° C for one hour with 100  $\mu$ Ci of <sup>51</sup>Cr in the presence or absence of 1  $\mu$ g/ml of the indicated peptides. Following this incubation, labeled P815 cells were washed to remove excess <sup>51</sup>Cr and peptide, and subsequently plated in duplicate in microculture plates at a concentration of 1,000 cells/well. Effector CTL (Chlamydia-specific CD8 T cells) were Following a 4 hour incubation, added at the indicated effector:target ratios. supernatants were harvested and measured by gamma-counter for release of 51Cr into the supernatant. Two overlapping peptides from the 298 amino acid open reading frame did specifically stimulate the CTL line. The peptides represented in SEQ ID NO: 138-156 were synthesized, representing the translation of the L2 homologue of the serovar D open reading frame for CT529 (Cap1 gene) and 216 amino acid open reading frame. As shown in Fig. 3, peptides CtC7.8-12 (SEQ ID NO: 18, also referred to as Cap1#132-147, SEQ ID NO: 139) and CtC7.8-13 (SEQ ID NO: 19, also referred to as Cap1#138-155, SEQ ID NO: 140) were able to elicit 38 to 52% specific lysis, respectively, at an effector to target ratio of 10:1. Notably, the overlap between these two peptides contained a predicted H2<sup>d</sup> (K<sup>d</sup> and L<sup>d</sup>) binding peptide. A 10 amino acid peptide was synthesized to correspond to this overlapping sequence (SEQ ID NO: 31) and was found to generate a strong immune response from the anti-Chlamydia CTL line by elispot assay. Significantly, a search of the most recent Genbank database revealed no

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proteins have previously been described for this gene. Therefore, the putative open reading frame encoding clone 2C7-8 (SEQ ID NO: 15) defines a gene which encompasses an antigen from *Chlamydia* capable of stimulating antigen-specific CD8+ T-cells in a MHC-I restricted manner, demonstrating this antigen could be used to develop a vaccine against *Chlamydia*.

To confirm these results and to further map the epitope, truncated peptides (SEQ ID NO: 138-156) were made and tested for recognition by the T-cells in an IFN-g ELISPOT assay. Truncations of either Ser139 (Cap1#140-147, SEQ ID NO: 146) or Leu147 (Cap1#138-146, SEQ ID NO: 147) abrogate T-cell recognition. These results indicate that the 9-mer peptide Cap1#139-147 (SFIGGITYL, SEQ ID NO: 145) is the minimal epitope recognized by the *Chlamydia*-specific T-cells.

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Sequence alignments of Cap1 (CT529) from selected serovars of C. trachomatis (SEQ ID NO: 121, 123, 125, 127, 129, 131, 133, 135, 137 and 139) shows one of the amino acid differences is found in position 2 of the proposed epitope. The homologous serovar D peptide is SIIGGITYL (SEQ ID NO: 168). The ability of SFIGGITYL and SIIGGITYL to target cells for recognition by the Chlamydia specific T-cells was compared. Serial dilutions of each peptide were incubated with P815 cells and tested for recognition by the T-cells in a 51Cr release assay, as described above. The Chlamydia-specific T-cells recognize the serovar L2 peptide at a minimum concentration of 1 nM and the serovar D peptide at a minimum concentration of 10 nM.

Further studies have shown that a Cap1#139-147-specific T-cell clone recognizes *C. trachomatis* infected cells. To confirm that Cap1<sub>139-147</sub> is presented on the surface of *Chlamydia* infected cells, Balb-3T3 (H-2<sup>d</sup>) cells were infected with *C. trachomatis* serovar L2 and tested to determine whether these cells are recognized by a CD8+ T-cell clone specific for Cap1#139-147 epitope (SEQ ID NO: 145). The T-cell clone specific for Cap1#139-147 epitope was obtained by limiting dilution of the line 69 T-cells. The T-cell clone specifically recognized the *Chlamydia* infected cells. In these experiments, target cells were *C. trachomatis* infected (positive control) or uninfected Balb/3T3 cells, showing 45%, 36% and 30% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively; or Cap1#139-147 epitope (SEQ ID NO: 145) coated, or untreated P815 cells, showing 83%, 75% and 58% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively (negative controls having less than 5% lysis in all cases). This data suggests that the epitope is presented during infection.

In vivo studies show Cap1#139-147 epitope-specific T-cells are primed during murine infection with *C. trachomatis*. To determine if infection with *C. trachomatis* primes a Cap1#139-147 epitope-specific T-cell response, mice were

infected i.p. with 10<sup>8</sup> IFU of *C. trachomatis* serovar L2. Two weeks after infection, the mice were sacrificed and spleen cells were stimulated on irradiated syngeneic spleen cells pulsed with Cap1#139-147 epitope peptide. After 5 days of stimulation, the cultures were used in a standard <sup>51</sup>Cr release assay to determine if there were Cap1#139-147 epitope-specific T-cells present in the culture. Specifically, spleen cells from a *C. trachomatis* serovar L2 immunized mouse or a control mouse injected with PBS after a 5 days culture with Cap1#139-147 peptide-coated syngeneic spleen cells and CD8+ T-cells able to specifically recognize Cap1#139-147 epitope gave 73%, 60% and 32% specific lysis at a30:1, 10:1 and 3:1 effector to target ratios, respectively. The control mice had a percent lysis of approximately 10% at a 30:1 effector to target ratio, and steadily declining with lowering E:T ratios. Target cells were Cap1#139-147 peptide-coated, or untreated P815 cells. These data suggest that Cap1#139-147 peptide-specific T-cells are primed during murine infection with *C. trachomatis*.

Studies were performed demonstrating that Ct529 (referred to herein as Cap-1) localizes to the inclusion membrane of *C. trachomatis*-infected cells and is not associated with elementary bodies or reticulate bodies. As described above, Cap-1 was identified as a product from *Chlamydia* that stimulates CD8+ CTL. These CTL are protective in a murine model of infection, thus making Cap-1 a good vaccine candidate. Further, since these CTL are MHC-I restricted, the Cap-1 gene must have access to the cytosol of infected cells, which may be a unique characteristic of specific *Chlamydial* gene products. Therefore, determination of the cellular localization of the gene products would be useful in characterizing Cap-1 as a vaccine candidate. To detect the intracellular localization of Cap-1, rabbit polyclonal antibodies directed against a recombinant polypeptide encompassing the N-terminal 125 amino acids of Cap-1 (SEQ ID NO: 305, with the amino acid sequence including the N-terminal 6-His tag provided in SEQ ID NO: 304) were used to stain McCoy cells infected with *Chlamydiae*.

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Rabbit-anti-Cap-1 polyclonal antibodies were obtained by hyper-immunization of rabbits with a recombinant polypeptide, rCt529c1-125 (SEQ ID NO: 305) encompassing the N-terminal portion of Cap-1. Recombinant rCt529e1-125 protein was obtained from *E. coli* transformed with a pET expression plasmid (as described above) encoding the nucleotides 1-375 encoding the N-terminal 1-125 amino acids of Cap-1. Recombinant protein was purified by Ni-NTA using techniques well known in the art. For a positive control antiserum, polyclonal antisera directed against elementary bodies were made by immunization of rabbits with purified *C. trachomatis* elementary bodies (Biodesign, Sacco, Maine). Pre-immune sera derived from rabbits prior to immunization with the Cap-1 polypeptide was used as a negative control.

Immunocytochemistry was performed on McCoy cell monolayers grown on glass coverslips inoculated with either C. trachomatis serovar L2 or C. psitacci, strain 6BC, at a concentration of 106 IFU (Inclusion Forming Units) per ml. After 2 hours, medium was aspirated and replaced with fresh RP-10 medium supplemented with cycloheximide (1.0 µg/ml). Infected cells were incubated at in 7% CO<sub>2</sub> for 24 hours and fixed by aspirating medium, rinsing cells once with PBS and methanol fixation for 5 minutes. For antigen staining, fixed cell monolayers were washed with PBS and incubated at 37°C for 2 hours with 1:100 dilutions of specific or control antisera. Cells were rinsed with PBS and incubated for 1 hour with fluorescein isothiocyanate (FITC)-labeled, anti-rabbit IgG (KPL, Gaithersburg) and stained with Evans blue (0.05%) in PBS. Fluorescence was observed with a 100X objective (Zeiss epifluorescence microscope), and photographed (Nikon UFX-11A camera).

Results from this study show Cap-1 localizes to the inclusion membrane of C. trachomatis-infected cells. Cap-1 specific antibody labeled the inclusion membranes of C. trachomatis-infected cells, but not Chlamydial elementary bodies contained in these inclusions or released by the fixation process. Conversely, the antielementary body antibody clearly labeled the bacterial bodies, not only within the inclusions, but those released by the fixation process. Specificity of the anti-Cap-1 antibody is demonstrated by the fact that it does not stain C. psittaci-infected cells. Specificity of the Cap-1 labeling is also shown by the absence of reactivity in preimmune sera. These results suggest that Cap-1 is released from the bacteria and becomes associated with the Chlamydial inclusion membrane. Therefore, Cap-1 is a gene product which may be useful for stimulating CD8+ T cells in the development of a vaccine against infections caused by Chlamydia.

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The relevance of the Cap-1 gene as a potential CTL antigen in a vaccine against Chlamydia infection is further illustrated by two additional series of studies. First, CTL specific for the MHC-I epitope of Cap-1 CT529 #138-147 peptide of C. trachomatis (SEQ ID NO: 144) have been shown to be primed to a high frequency during natural infection. Specifically, Balb/C mice were inoculated with 106 I.F.U. of 30 C. trachomatis, serova L2. After 2 weeks, spleens were harvested and quantified by Elispot analysis for the number of IFN-γ secreting cells in response to Cap-1 #138-147 peptide-pulsed antigen presenting cells. In two experiments, the number of IFN-ysecreting cells in 105 splenocytes was about 1% of all CD8+ T-cells. This high frequency of responding CD8+ CTL to the MHC-1 epitope (Cap-1 CT529 #138-147 peptide) suggest that Cap-1 is highly immunogenic in infections.

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Results from a second series of studies have shown that the Cap-1 protein is almost immediately accessible to the cytosol of the host cell upon infection. This is shown in a time-course of Cap-1 CT529 #138-147 peptide presentation. Briefly, 3T3 cells were infected with *C. trachomatis* serovar L2 for various lengths of time, and then tested for recognition by Cap-1 CT529 #138-147 peptide-specific CTL. The results show that *C. trachomatis*-infected 3T3 cells are targeted for recognition by the antigen-specific CTL after only 2 hours of infection. These results suggest that Cap-1 is an early protein synthesized in the development of *C. trachomatis* elementary bodies to reticulate bodies. A CD8+ CTL immune response directed against a gene product expressed early in infection may be particularly efficacious in a vaccine against *Chlamydia* infection.

# EXAMPLE 5

# GENERATION OF ANTIBODY AND T-CELL RESPONSES IN MICE IMMUNIZED WITH CHLAMYDIA ANTIGENS

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Immunogenicity studies were conducted to determine the antibody and CD4+ T cell responses in mice immunized with either purified SWIB or S13 proteins formulated with Montanide adjuvant, or DNA-based immunizations with pcDNA-3 expression vectors containing the DNA sequences for SWIB or S13. SWIB is also referred to as clone 1-B1-66 (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5), and S13 ribosomal protein is also referred to as clone 10-C10-31 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12). In the first experiment, groups of three C57BL/6 mice were immunized twice and monitored for antibody and CD4+ T-cell responses. DNA immunizations were intradermal at the base of the tail and polypeptide immunizations were administered by subcutaneous route. Results from standard <sup>3</sup>H-incorporation assays of spleen cells from immunized mice shows a strong proliferative response from the group immunized with purified recombinant SWIB polypeptide (SEQ ID NO: 5). Further analysis by cytokine induction assays, as previously described, demonstrated that the group immunized with SWIB polypeptide produced a measurable IFN-y and IL-4 response. Subsequent ELISA-based assays to determine the predominant antibody isotype response in the experimental group immunized with the SWIB polypeptide were performed. Fig. 4 illustrates the SWIB-immunized group gave a humoral response that was predominantly IgG1.

In a second experiment, C3H mice were immunized three times with 10 µg purified SWIB protein (also referred to as clone 1-B1-66, SEQ ID NO: 5)

formulated in either PBS or Montanide at three week intervals and harvested two weeks after the third immunization. Antibody titers directed against the SWIB protein were determined by standard ELISA-based techniques well known in the art, demonstrating the SWIB protein formulated with Montanide adjuvant induced a strong humoral immune response. T-cell proliferative responses were determined by a XTT-based assay (Scudiero, et al, *Cancer Research*, 1988, 48:4827). As shown in Fig. 5, splenocytes from mice immunized with the SWIB polypeptide plus Montanide elicited an antigen specific proliferative response. In addition, the capacity of splenocytes from immunized animals to secrete IFN-γ in response to soluble recombinant SWIB polypeptide was determined using the cytokine induction assay previously described. The splenocytes from all animals in the group immunized with SWIB polypeptide formulated with montanide adjuvant secreted IFN-γ in response to exposure to the SWIB Chlamydia antigen, demonstrating an *Chlamydia*-specific immune response.

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In a further experiment, C3H mice were immunized at three separate time points at the base of the tail with 10 μg of purified SWIB or S13 protein (*C. trachomatis*, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) formulated with the SBAS2 adjuvant (SmithKline Beecham, London, England). Antigen-specific antibody titers were measured by ELISA, showing both polypeptides induced a strong IgG response, ranging in titers from 1 x10<sup>-4</sup> to 1 x10<sup>-5</sup>. The IgG1 and IgG2a components of this response were present in fairly equal amounts. Antigen-specific T-cell proliferative responses, determined by standard <sup>3</sup>H-incorporation assays on spleen cells isolated from immunized mice, were quite strong for SWIB (50,000 cpm above the negative control) and even stronger for s13 (100,000 cpm above the negative control). The IFNγ production was assayed by standard ELISA techniques from supernatant from the proliferating culture. *In vitro* restimulation of the culture with S13 protein induced high levels of IFNγ production, approximately 25 ng/ml versus 2 ng/ml for the negative control. Restimulation with the SWIB protein also induced IFNγ, although to a lesser extent.

In a related experiment, C3H mice were immunized at three separate time points with 10 µg of purified SWIB or S13 protein (*C. trachomatis*, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) mixed with 10 µg of Cholera Toxin. Mucosal immunization was through intranasal inoculation. Antigen-specific antibody responses were determined by standard ELISA techniques. Antigen-specific IgG antibodies were present in the blood of SWIB-immunized mice, with titers ranging from 1 x10<sup>-3</sup> to 1 x10<sup>-4</sup>, but non-detectable in the S13-immunized animals. Antigen-specific T-cell responses from isolated splenocytes,

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as measured by IFN $\gamma$  production, gave similar results to those described immediately above for systemic immunization.

An animal study was conducted to determine the immunogenicity of the CT529 serovar LGVII CTL epitope, defined by the CT529 10mer consensus peptide (CSFIGGITYL - SEQ ID NO: 31), which was identified as an H2-Kd restricted CTL epitope. BALB/c mice (3 mice per group) were immunized three times with 25 µg of peptide combined with various adjuvants. The peptide was administered systemically at the base of the tail in either SKB Adjuvant System SBAS-2", SBAS-7 (SmithKline Beecham, London, England) or Montanide. The peptide was also administered intranasally mixed with 10ug of Cholera Toxin (CT). Naive mice were used as a control. Four weeks after the 3rd immunization, spleen cells were restimulated with LPS-blasts pulsed with 10ug/ml CT529 10mer consensus peptide at three different effector to LPS-blasts ratios: 6, 1.5 and 0.4 at 1x106 cell/ml. After 2 restimulations, effector cells were tested for their ability to lyse peptide pulsed P815 cells using a standard chromium release assay. A non-relevant peptide from chicken egg ovalbumin was used as a negative control. The results demonstrate that a significant immune response was elicited towards the CT529 10mer consensus peptide and that antigenspecific T-cells capable of lysing peptide-pulsed targets were elicited in response to immunization with the peptide. Specifically, antigen-specific lytic activities were found in the SBAS-7 and CT adjuvanted group while Montanide and SBAS-2" failed to adjuvant the CTL epitope immunization.

# **EXAMPLE 6**

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# EXPRESSION AND CHARACTERIZATION OF CHLAMYDIA PNEUMONIAE GENES

The human T-cell line, TCL-8, described in Example 1, recognizes Chlamydia trachomatis as well as Chlamydia pneumonia infected monocyte-derived dendritic cells, suggesting Chlamydia trachomatis and pneumonia may encode cross-reactive T-cell epitopes. To isolate the Chlamydia pneumonia genes homologous to Chlamydia trachomatis LGV II clones 1B1-66, also referred to as SWIB (SEQ ID NO: 1) and clone 10C10-31, also referred to as S13 ribosomal protein (SEQ ID NO: 4), HeLa 229 cells were infected with C. pneumonia strain TWAR (CDC/CWL-029). After three days incubation, the C. pneumonia-infected HeLa cells were harvested, washed and resuspended in 200 µl water and heated in a boiling water bath for 20 minutes. Ten microliters of the disrupted cell suspension was used as the PCR template.

C. pneumonia specific primers were designed for clones 1B1-66 and 10C10-31 such that the 5' end had a 6X-Histidine tag and a Nde I site inserted, and the

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3' end had a stop codon and a BamHI site included (Fig. 6). The PCR products were amplified and sequenced by standard techniques well known in the art. The C. pneumonia-specific PCR products were cloned into expression vector pET17B (Novagen, Madison, WI) and transfected into E. coli BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel chromatographic methodology provided by Novagen. Two proteins from C. pneumonia were thus generated, a 10-11 kDa protein referred to as CpSWIB (SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 28, respectively), a 15 kDa protein referred to as CpS13 (SEQ ID NO: 29, and SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 30 and 91, respectively).

### **EXAMPLE 7**

# INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y PRODUCTION BY CHLAMYDIA PNEUMONIAE ANTIGENS

The ability of recombinant *Chlamydia pneumoniae* antigens to induce T cell proliferation and interferon-y production is determined as follows.

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Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatography (Webb et al., *J. Immunology 157*:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from *C. pneumoniae* patients as well as from normal donors whose T-cells are known to proliferate in response to *Chlamydia* antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 µg/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 µg/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 µl, 50 µl of medium is removed from each well for determination of IFN-γ levels, as described below. The plates are then pulsed with 1 µCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at

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room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

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A human anti-Chlamydia T-cell line (TCL-8) capable of cross-reacting to C. trachomatis and C. pneumonia was used to determine whether the expressed proteins described in the example above, (i.e., CpSWIB, SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 28, respectively, and the 15 kDa protein referred to as CpS13 SEQ ID NO: 29, and SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 30 and 91, respectively), possessed T-cell epitopes common to both C. trachomatis and C. pneumonia. Briefly, E. coli expressing Chlamydial proteins were titered on 1 x 104 monocyte-derived dendritic cells. After two hours, the dendritic cells cultures were washed and 2.5 x 104 T cells (TCL-8) added and allowed to incubate for an additional 72 hours. The amount of INF-y in the culture supernatant was then determined by ELISA. As shown in Figs. 7A and 7B, the TCL-8 T-cell line specifically recognized the S13 ribosomal protein from both C. trachomatis and C. pneumonia as demonstrated by the antigen-specific induction of IFN-y, whereas only the SWIB protein from C. trachomatis was recognized by the T-cell line. To validate these results, the T cell epitope of C. trachomatis SWIB was identified by epitope mapping using target cells pulsed with a series of overlapping peptides and the T-cell line TCL-8. 3H-thymidine incorporation assays demonstrated that the peptide, referred to as C.t.SWIB 52-67, of SEQ ID NO: 39 gave the strongest proliferation of the TCL-8 line. The homologous peptides corresponding to the SWIB of C. pneumoniae sequence (SEQ ID NO: 40), the topoisomerase-SWIB fusion of C. pneumoniae (SEQ ID NO: 43) and C. trachomatis (SEQ ID NO: 42) as well as the human SWI domain (SEQ ID NO: 41) were synthesized and tested in the above assay. The T-cell line TCL-8 only recognized the C. trachomatis peptide of SEQ ID NO: 39 and not the corresponding C. pneumoniae peptide (SEQ ID NO: 40), or the other corresponding peptides described above (SEQ ID NO; 41-43).

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Chlamydia-specific T cell lines were generated from donor CP-21 with a positive serum titer against *C. pneumoniae* by stimulating donor PBMC with either *C. trachomatis* or *C. pneumoniae*-infected monocyte-derived dendritic cells, respectively. T-cells generated against *C. pneumoniae* responded to recombinant *C. pneumoniae*-SWIB but not *C. trachomatis*-SWIB, whereas the T-cell line generated against *C. trachomatis* did not respond to either *C. trachomatis*- or *C. pneumoniae*-SWIB (see Fig. 9). The *C. pneumoniae*-SWIB specific immune response of donor CP-21 confirms the *C. pneumoniae* infection and indicates the elicitation of *C. pneumoniae*-SWIB specific T-cells during *in vivo C. pneumoniae* infection.

Epitope mapping of the T-cell response to *C. pneumoniae*-SWIB has shown that Cp-SWIB-specific T-cells responded to the overlapping peptides Cp-SWIB 32-51 (SEQ ID NO: 101) and Cp-SWIB 37-56 (SEQ ID NO: 102), indicating a *C. pneumoniae*-SWIB-specific T-cell epitope Cp-SWIB 37-51 (SEQ ID NO: 100).

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In additional experiments, T-cell lines were generated from donor CP1, also a C. pneumoniae seropositive donor, by stimulating PBMC with non-infectious elementary bodies from C. trachomatis and C. pneumoniae, respectively. In particular, proliferative responses were determined by stimulating 2.5 x 10<sup>4</sup> T-cells in the presence of 1 x 104 monocyte-derived dendritic cells and non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or either recombinant C. trachomatis or C. pneumoniae SWIB protein. The T-cell response against SWIB resembled the data obtained with T-cell lines from CP-21 in that C. pneumoniae-SWIB, but not C. trachomatis-SWIB elicited a response by the C. pneumoniae T-cell line. In addition, the C. trachomatis T-cell line did not proliferate in response to either C. trachomatis or C. pneumoniae SWIB, though it did proliferate in response to both CT and CP elementary bodies. As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 10<sup>4</sup> TCP-21 T-cells in the presence of 1 x 10<sup>4</sup> monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP- 10

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21 T-cell line also gave a proliferative response to the homologous *C. pneumoniae* peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the to the *C. trachomatis* peptides. The amino acid substitutions in position two (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between *C. trachomatis* and *C. pneumoniae*.

# **EXAMPLE 8**

# IMMUNE RESPONSES OF HUMAN PBMC AND T-CELL LINES

# AGAINST CHLAMYDIA ANTIGENS

The examples provided herein suggest that there is a population of healthy donors among the general population that have been infected with C. trachomatis and generated a protective immune response controlling the C. trachomatis infection. These donors remained clinically asymptomatic and seronegative for C. To characterize the immune responses of normal donors against trachomatis. chlamydial antigens which had been identified by CD4 expression cloning, PBMC obtained from 12 healthy donors were tested against a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and C. trachomatis-, C. pneumoniae-S13. The data are summarized in Table I below. All donors were seronegative for C. trachomatis, whereas 6/12 had a positive C. pneumoniae titer. Using a stimulation index of >4 as a positive response, 11/12 of the subjects responded to C. trachomatis elementary bodies and 12/12 responded to C. pneumoniae elementary bodies. One donor, AD104, responded to recombinant C. pneumoniae-S13 protein, but not to recombinant C. trachomatis-S13 protein, indicating a C. pneumoniae-specific Three out of 12 donors had a C. trachomatis-SWIB, but not a C. pneumoniae-SWIB specific response, confirming a C. trachomatis infection. C. trachomatis and C. pneumoniae-S13 elicited a response in 8/12 donors suggesting a chlamydial infection. These data demonstrate the ability of SWIB and S13 to elicit a Tcell response in PBMC of normal study subjects.

89 TABLE I

Immune response of normal	study subject	s against Chlamydia
immune response of normal	Study Subject	s agamsi Cimaniyaia

Donor	Sex	<i>Chlamydia</i> IgG titer	CT EB	CP EB	CT Swib	CP Swib	CT S13	CP S13	CT lpdA	CT TSA
AD100	male	negative	++	+++	+	-	++	++	-	n.t.
AD104	female	negative	+++	++	-	-	•	++	· -	n.t.
AD108	male	CP 1:256	++	++	+	+/-	+	+	+	n.t.
AD112	female	negative	++	++	+	-	+	-	+/-	n.t.
AD120	male	negative	-	+	•	-	-	-	-	n.t.
AD124	female	CP 1:128	++	++	-	-	-	-	-	n.t.
AD128	male	CP 1:512	+	++	-	-	++	+	++	-
AD132	female	negative	++	++	-	-	+	+	-	-
AD136	female	CP 1:128	+	++	-	-	+/-	-	-	-
AD140	male	CP 1:256	++	++	-	-	+	+	-	-
AD142	female	CP 1:512	++	++	•	-	+	+	+	-
AD146	female	negative	++	++			++	+	+	

CT= Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant Chlamydia TSA protein. Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating 3 x 10<sup>5</sup> PBMC with 1 x 10<sup>4</sup> monocyte-derived dendritic cells pre-incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a <sup>3</sup>H-thymidine pulse for the last 18h.

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SI: Sti	mulation index	
+/-:	SI ~	4
+:	SI >	4

SI 10-30 +++: SI> 30

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In a first series of experiments, T-cell lines were generated from a healthy female individual (CT-10) with a history of genital exposure to C. trachomatis by stimulating T-cells with C. trachomatis LGV II elementary bodies as previously described. Although the study subject was exposed to C. trachomatis, she did not seroconvert and did not develop clinical symptoms, suggesting donor CT-10 may have developed a protective immune response against C. trachomatis. As shown in Fig. 10, a primary Chlamydia-specific T-cell line derived from donor CT-10 responded to C. trachomatis-SWIB, but not C. pneumoniae-SWIB recombinant proteins, confirming the exposure of CT-10 to C. trachomatis. Epitope mapping of the T-cell response to C. 15 trachomatis-SWIB showed that this donor responded to the same epitope Ct-SWIB 52-67 (SEQ ID NO: 39) as T-cell line TCL-8, as shown in Fig. 11.

Additional T-cell lines were generated as described above for various C. trachomatis patients. A summary of the patients' clinical profile and proliferative responses to various C. trachomatis and C. pneumoniae elementary bodies and recombinant proteins are summarized in Table II.

TABLE II

Proliferative response of C. trachomatis patients										
Patients	Clinical manifestation	IgG titer	CT EB	CP EB	CT Swib	CP Swib	CT S13	CP S13	CT IpdA	CT TSA
CT-1	NGU	педатіче	+	+	_	-	++	++	++	+
CT-2	NGU	negative	++	++	-	-	+	+/-	-	-
CT-3	asymptomatic shed Eb Dx was HPV	Ct 1:512 Cp 1:1024 Cps 1:256	+	+	-	-	+	-	+	-
CT-4	asymptomatic shed Eb	Ct 1:1024	+	+	-	-	-	-	-	-
CT-5	BV	Ct 1:256 Cp 1:256	++	++	-	-	+	-	-	-
CT-6	perinial rash discharge	Cp 1:1024	+	+	-	-	-	-	-	-
CT-7	BV genital ulcer	Ct 1:512 Cp 1:1024	+	+	•	-	+	+	+	-
<b>CT-8</b>	Not known	Not tested	++	++	-	-	-	-	-	-
CT-9	asymptomatic	Ct 1:128 Cp 1:128	+++	++	-		++	+	· <del>+</del>	<u>.</u>
CT-10	Itch mild vulvar	negative	++	++	-	-	-	-	-	-
CT-11	BV, abnormal pap	Ct 1: 512	+++	+++	-	•	+++	+/-	++	+
CT-12	asymptomatic	Cp 1: 512	++	++	-	-	++	+	+	-

NGU= Non-Gonococcal Urethritis; BV= Bacterial Vaginosis; CT=
Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary
bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia
S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant
Chlamydia TSA protein

Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating  $3 \times 10^5$  PBMC with  $1 \times 10^4$  monocyte-

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derived dendritic cells pre-incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a <sup>3</sup>H-thymidine pulse for the last 18 hours.

5 SI: Stimulation index

+/-: SI ~ 4 +: SI > 4 ++: SI 10-30 +++: SI > 30

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Using the panel of asymptomatic (as defined above) study subjects and C. trachomatis patients, as summarized in Tables I and II, a comprehensive study of the immune responses of PBMC derived from the two groups was conducted. Briefly, PBMCs from C. pneumoniae patients as well as from normal donors are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50  $\mu$  g/ml gentamicin. Purified polypeptides, a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and S13, as well as . C. trachomatis lpdA and TSA are added in duplicate at concentrations of 0.5 to 10  $\mu$ g/mL. After six days of culture in 96-well round-bottom plates in a volume of 200  $\mu$ l, 50  $\mu$ l of medium is removed from each well for determination of IFN- $\gamma$  levels, as described below. The plates are then pulsed with 1  $\mu$ Ci/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

Proliferative responses to the recombinant Chlamydiae antigens demonstrated that the majority of asymptomatic donors and C. trachomatis patients recognized the C. trachomatis S13 antigen (8/12) and a majority of the C. trachomatis patients recognized the C. pneumonia S13 antigen (8/12), with 4/12 asymptomatic donors also recognizing the C. pneumonia S13 antigen. Also, six out of twelve of the C. trachomatis patients and four out of twelve of the asymptomatic donors gave a proliferative response to the lpdA antigen of C. trachomatis. These results demonstrate that the C. trachomatis and C. pneumonia S13 antigen, C. trachomatis Swib antigen and the C. trachomatis lpdA antigen are recognized by the asymptomatic donors, indicating these antigens were recognized during exposure to Chlamydia and an immune response elicited against them. This implies these antigens may play a role in conferring protective immunity in a human host. In addition, the C. trachomatis patients, pneumonia S13 antigen is recognized equally well among the C. trachomatis patients,

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therefore indicating there may be epitopes shared between C. trachomatis and C. pneumonia in the S13 protein. Table III summarizes the results of these studies.

**TABLE III** 

	NORMAL DONORS	C.T. PATIENTS
A. Antigen		
C.tSwib	3/12	0/12
C.pSwib	0/12	0/12
C.tS13	8/12	8/12
C.pS13	4/12	8/12
lpdA	4/12	6/12
TSA	0/12	2/12

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A series of studies were initiated to determine the cellular immune response to short-term T-cell lines generated from asymptomatic donors and C. Cellular immune responses were measured by standard trachomatis patients. proliferation assays and IFN-y, as described in Example 7. Specifically, the majority of the antigens were in the form of single E. coli clones expressing Chlamydial antigens, although some recombinant proteins were also used in the assays. The single E. coli clones were titered on 1 x 104 monocyte-derived dendritic cells and after two hours, the culture was washed and 2.5 x 10<sup>4</sup> T-cells were added. The assay using the recombinant proteins were performed as previously described. Proliferation was determined after four days with a standard <sup>3</sup>H-thymidine pulse for the last 18 hours. Induction of IFN-γ , was determined from culture supernatants harvested after four days using standard ELISA assays, as described above. The results show that all the C. trachomatis antigens tested, except for C.T. Swib, elicited a proliferative response from one or more different T-cell lines derived form C. trachomatis patients. In addition, proliferative responses were elicited from both the C. trachomatis patients and asymptomatic donors for the following Chlamydia genes, CT622, groEL, pmpD, CT610 and rS13.

The 12G3-83 clone also contains sequences to CT734 and CT764 in addition to CT622, and therefore these gene sequence may also have immunoreactive epitopes. Similarly, clone 21G12-60 contains sequences to the hypothetical protein genes CT229 and CT228 in addition to CT875; and 15H2-76 also contains sequences

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from CT812 and CT088, as well as sharing homology to the sycE gene. Clone 11H3-61 also contains sequences sharing homology to the PGP6-D virulence protein.

TABLE IV

Clone	C. t. Antigen (putative*)	TCL from Asymp. Donors	TCL from C. t. Patients	SEQ ID NO:
1B1-66 (E. coli)	Swib	2/2	0/4	5
1B1-66 (protein)	Swib	2/2	. 0/4	5
12G3-83 (E. coli)	CT622*	2/2	4/4	57
22B3-53 (E. coli)	GROEL	1/2	. 4/4	111
22B3-53 (protein)	GROEL	1/2	4/4	111
15H2-76 (E. coli)	PMPD*	1/2	3/4	87
11H3-61 (E. coli)	rL1*	0/2	3/4	60
14H1-4 (E. coli)	TSA	0/2	3/4	56
14H1-4 (protein)	TSA	0/2	3/4	56
11G10-46 (E. coli)	CT610	1/2	1/4	62
10C10-17 (E. coli)	rS13	1/2	1/4	62
10C10-17 (protein)	RS13	1/2	1/4	62
21G12-60 (E. coli)	CT875*	0/2	2/4	110
11H4-32 (E. coli)	DNAK	0/2	2/4	59
21C7-8 (E. coli)	DNAK	0/2	2/4	115
17C10-31 (E. coli)	CT858	0/2	2/4	114

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# **EXAMPLE 9**

# PROTECTION STUDIES USING CHLAMYDIA ANTIGENS

Protection studies were conducted in mice to determine whether immunization with chlamydial antigens can impact on the genital tract disease resulting from chlamydial inoculation. Two models were utilized; a model of intravaginal inoculation that uses a human isolate containing a strain of *Chlamydia psittaci* (MTW447), and a model of intrauterine inoculation that involves a human isolate identified as *Chlamydia trachomatis*, serovar F (strain NI1). Both strains induce inflammation in the upper genital tract, which resemble endometritis and salpingitis caused by *Chlamydia trachomatis* in women. In the first experiment, C3H mice (4)

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mice per group) were immunized three times with 100 µg of pcDNA-3 expression vector containing C. trachomatis SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5). Inoculations were at the base of the tail for systemic immunization. Two weeks after the last immunization, animals were progesterone treated and infected, either thru the vagina or by injection of the inoculum in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored (from + for very mild, to +++++ for very severe). Scores attributed to each single oviduct /ovary were summed and divided by the number of organs examined to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control-immunized animals receiving empty vector showed consistent inflammation with an ovary /oviduct mean inflammation score of 6.12, in contrast to 2.62 for the DNA-immunized group. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 8.37, versus 5.00 for the DNA-immunized group. Also, in the later model, vaccinated mice showed no signs of tubal occlusion while negative control vaccinated groups had inflammatory cells in the lumen of the oviduct

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In a second experiment, C3H mice (4 mice per group) were immunized three times with 50 µg of pcDNA-3 expression vector containing *C. trachomatis* SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5) encapsulated in Poly Lactide co-Glycolide microspheres (PLG); immunizations were made intra-peritoneally. Two weeks after the last immunization, animal were progesterone treated and infected by inoculation of *C. psittaci* in the vagina. Two weeks after infection, mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored as previously described. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean of inflammation for the group. Negative control-immunized animals receiving PLG-encapsulated empty vector showed consistent infammation with an ovary /oviduct mean inflammation score of 7.28, versus 5.71 for the PLG-encapsulated DNA immunized group. Inflammation in the peritoneum was 1.75 for the vaccinated group versus 3. 75 for the control.

In a third experiment, C3H mice (4 per group) were immunized three times with 10 µg of purified recombinant protein, either SWIB (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5, or S13 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12) mixed with Cholera Toxin (CT); the preparation was administred intranasally upon anaesthesia

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in a 20 uL volume. Two weeks after the last immunization, animal were progesterone treated and infected, either by vaginal inoculation of *C. psittaci* or by injection of *C. trachomatis* serovar F in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. The degree of inflammation was scored as described above. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control- immunized animals receiving cholera toxin alone showed an ovary /oviduct mean inflammation score of 4.25 (only 2 mice analyzed; 2 other died) versus 5.00 for the s13 plus cholera toxin-immunized group, and 1.00 for the SWIB plus cholera toxin. Untreated infected animals had an ovary /oviduct mean inflammation score of 7. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 7.37 versus 6.75 for the s13 plus cholera toxin-immunized group and 5.37 for the SWIB plus cholera toxin-immunized group. Untreated infected animals had an ovary /oviduct mean inflammation score of 8.

The three experiments described above suggest that SWIB-specific protection is obtainable. This protective effect is more marked in the model of homologous infection but is still present when in a heterologous challenge infection with *C. psittaci*.

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## EXAMPLE 10

### PMP/RA12 Fusion Proteins

Various Pmp/Ra12 fusion constructs were generated by first synthesizing PCR fragments of a Pmp gene using primers containing a Not I restriction site. Each PCR fragment was then ligated into the Notl restriction site of pCRX1. The pCRX1 vector contains the 6HisRa12 portion of the fusion. The Ra12 portion of the fusion construct encodes a polypeptide corresponding to amino acid residues 192-323 of *Mycobacterium tuberculosis* MTB32A, as described in U.S. Patent Application 60/158,585, the disclosure of which is incorporated herein by reference. The correct orientation of each insert was determined by its restriction enzyme pattern and its sequence was verified. Multiple fusion constructs were made for PmpA, PmpB, PmpC, PmpF and PmpH, as described further below:

### PMPA FUSION PROTEINS

PmpA is 107 kD protein containing 982 aa and was cloned from serovar E. The PmpA protein was divided into 2 overlapping fragments, the PmpA(N-terminal) and (C-terminal) portions.

PmpA(N-term) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCATGTTTATAACAAAGGAACTTATG (SEQ ID NO:306)

GAGAGCGGCCGCTTACTTAGGTGAGAAGAAGGGAGTTTC (SEQ ID NO:307)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 308, encoding a 66 kD protein (619aa) expressing the segment 1-473 aa of PmpA. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 309.

PmpA(C-term) was amplified by the sense and antisense primers:

15 GAGAGCGGCCGCTCCATTCTATTCATTTCTTTGATCCTG (SEQ ID NO:310)

GAGAGCGGCCGCTTAGAAGCCAACATAGCCTCC (SEQ ID NO:311)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 312, encoding a 74 kD protein (691aa) expressing the segment 438-982 aa of PmpA. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 313.

# PMPF FUSION PROTEINS

PmpF is 112 kD protein containing 1034 aa and was cloned from the serovar E. PmpF protein was divided into 2 overlapping fragments, the PmpF(N- term) and (C-term) portions.

PmpF(N-term) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCATGATTAAAAGAACTTCTCTATCC (SEQ ID NO:314)

30 GAGAGCGGCCGCTTATAATTCTGCATCATCTTCTATGGC (SEQ ID NO:315)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 316, encoding a 69 kD protein (646aa) expressing the segment 1-499 aa of PmpF. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 317.

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PmpF(C-term) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGACATACGAACTCTGATGGG (SEQ ID NO:318)

. GAGAGCGGCCGCTTAAAAGACCAGAGCTCCTCC (SEQ ID NO:319)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 320, encoding a 77 kD protein (715aa) expressing the segment 466-1034aa of PmpF. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 321.

10 PMPH FUSION PROTEINS

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PmpH is 108 kD protein containing 1016 aa and was cloned from the serovar E. PmpH protein was divided into 2 overlapping fragments, the PmpH(N-term)and (C-term)portions.

PmpH(N-term) was amplified by the sense and antisense primers:

15 GAGAGCGGCCGCTCATGCCTTTTTCTTTGAGATCTAC (SEQ ID NO:322)

GAGAGCGGCCGCTTACACAGATCCATTACCGGACTG (SEQ ID NO:323)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 324, encoding a 64 kD protein (631aa) expressing the segment 1-484 aa of PmpH. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 325.

PmpH(C-term) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGATCCTGTAGTACAAAATAATTCAGC (SEQ ID NO:326)

25 GAGAGCGGCCGCTTAAAAGATTCTATTCAAGCC (SEQ ID NO:327)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 328, encoding a 77 kD protein (715aa) expressing the segment 449-1016aa of PmpH. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 329.

PMPB FUSION PROTEINS

PmpB is 183 kD protein containing 1750 as and was cloned from the serovar E. PmpB protein was divided into 4 overlapping fragments, PmpB(1), (2), (3) and (4).

PmpB(1) was amplified by the sense and antisense primers:

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GAGAGCGGCCGCTCATGAAATGGCTGTCAGCTACTGCG (SEQ ID NO:330)

GAGAGCGGCCGCTTACTTAATGCGAATTTCTTCAAG (SEQ ID NO:331)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 332, and encodes is a 53 kD protein (518aa) expressing the segment 1-372 aa of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 333.

PmpB(2) was amplified by the sense and antisense primers:

GAGAGCGCCCCCCGGTGACCTCTCAATTCAATCTTC (SEQ ID NO:334)

GAGAGCGGCCGCTTAGTTCTCTGTTACAGATAAGGAGAC (SEQ ID NO:335)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 336 and encodes a 60 kD protein (585aa) expressing the segment 330-767 aa of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 337.

PmpB(3) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGACCAACTGAATATCTCTGAGAAC (SEQ ID NO:338)

GAGCGGCCGCTTAAGAGACTACGTGGAGTTCTG (SEQ ID NO:339)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 340 encodes a 67 kD protein (654aa) expressing the segment 732-1236 aa of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 341

PmpB(4) was amplified by the sense and antisense primers:

25 GAGAGCGGCCGCTCGGAACTATTGTGTTCTCTTCTG (SEQ ID NO:342)

GAGAGCGGCCGCTTAGAAGATCATGCGAGCACCGC (SEQ ID NO:343)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 344 encodes a 76 kD protein (700aa) expressing the segment 1160-1750 of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 345.

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## **PMPC FUSION PROTEINS**

PmpC is 187 kD protein containing 1774 aa and was cloned from the serovar E/L2. PmpC protein was divided into 3 overlapping fragments, PmpC(1), (2) and (3).

PmpC(1) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCATGAAATTTATGTCAGCTACTGC (SEQ ID NO:346)

GAGAGCGGCCGCTTACCCTGTAATTCCAGTGATGGTC (SEQ ID NO:347)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 348 and encodes a 51 kD protein (487aa) expressing the segment 1-340 aa of PmpC. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 349.

PmpC(2) was amplified by the sense and antisense primers:

15 GAGAGCGGCCGCTCGATACACAAGTATCAGAATCACC (SEQ ID NO:350)

GAGAGCGGCCGCTTAAGAGGACGATGAGACACTCTCG (SEQ ID NO:351)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 352 and encodes a 60 kD protein (583aa) expressing the segment 305-741 aa of PmpC. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 353.

PmpC(3) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGATCAATCTAACGAAAACACAGACG (SEQ ID NO:354)

25 GAGAGCGGCCGCTTAGACCAAAGCTCCATCAGCAAC (SEQ ID NO:355)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 356 and encodes a 70 kD protein (683aa) expressing the segment 714-1250 aa of PmpC. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 357.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

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# **CLAIMS**

- 1. An isolated polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-290; (b) sequences complementary to a sequence of (a); and (c) polynucleotide sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
  - 2. The polypeptide of claim 1 wherein the polypeptide comprises a sequence selected from the group consisting of SEQ ID NO: 175-180, 189-196, 264 and 266.
  - 3. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1 and 2.
  - 4. A recombinant expression vector comprising a polynucleotide molecule according to claim 3.
    - 5. A host cell transformed with an expression vector according to claim 4.
  - 6. The host cell of claim 5 wherein the host cell is selected from the group consisting of *E. coli*, yeast and mammalian cells.
  - 7. A fusion protein comprising a polypeptide according to any one of claims 1 and 2.
  - 8. A fusion protein according to claim 7, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell transfected with a polynucleotide encoding the fusion protein.
  - 9. A fusion protein according to claim 7, wherein the fusion protein comprises a T helper epitope that is not present within the polypeptide of claim 1.
  - 10. A fusion protein according to claim 7, wherein the fusion protein comprises an affinity tag.

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- 11. An isolated polynucleotide encoding a fusion protein according to claim 7.
- 12. An isolated monoclonal antibody, or antigen-binding fragment thereof, that specifically binds to a Chlamydia protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence according to claim 1, or a complement of any of the foregoing polynucleotide sequences.
- 13. A pharmaceutical composition comprising a polypeptide according to claim 1, and a physiologically acceptable carrier.
- 14. A pharmaceutical composition comprising a polynucleotide molecule according to claim 3 and a physiologically acceptable carrier.
- 15. A pharmaceutical composition comprising a polypeptide and a physiologically acceptable carrier, wherein the polypeptide is encoded by polynucleotide molecule selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 16. A pharmaceutical composition comprising a polynucleotide molecule and a physiologically acceptable carrier, wherein the polynucleotide molecule comprises a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 17. A pharmaceutical composition comprising a physiologically acceptable carrier and at least one component selected from the group consisting of:
  - (a) a fusion protein according to claim 7;
  - (b) a polynucleotide according to claim 11; and
  - (c) an antibody according to claim 12.
- 18. A vaccine comprising a polypeptide according to claim 1, and an immunostimulant.

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- 19. A vaccine comprising a polynucleotide molecule according to claim 3 and an immunostimulant.
- 20. A vaccine comprising a polypeptide and an immunostimulant, wherein the polypeptide is encoded by a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 21. A vaccine comprising a DNA molecule and an immunostimulant, wherein the DNA molecule comprises a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 22. A vaccine comprising an immunostimulant and at least one component selected from the group consisting of:
  - (a) a fusion protein according to claim 7;
  - (b) a polynucleotide according to claim 11; and
  - (c) an antibody according to claim 12.
- 23. The vaccine of any one of claims 18-22 wherein the immunostimulant is an adjuvant.
- 24. A method for inducing protective immunity in a patient, comprising administering to a patient a pharmaceutical composition according to any one of claims 13-17.
- 25. A method for inducing protective immunity in a patient, comprising administering to a patient a vaccine according to any one of claims 18-22.
- 26. An isolated polyclonal antibody, or antigen-binding fragment thereof, that specifically binds to a Chlamydia protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence according to claim 1, or a complement of any of the foregoing polynucleotide sequences.

- 27. A method for detecting Chlamydia infection in a patient, comprising:
- (a) obtaining a biological sample from the patient;
- (b) contacting the sample with a polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291. (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
  - (c) detecting the presence of antibodies that bind to the polypeptide.
  - 28. A method for detecting *Chlamydia* infection in a patient, comprising:
  - (a) obtaining a biological sample from the patient;
  - (b) contacting the sample with a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
    - (c) detecting the presence of antibodies that bind to the fusion protein.
  - 29. The method of any one of claims 27 and 28 wherein the biological sample is selected from the group consisting of whole blood, serum, plasma, saliva, cerebrospinal fluid and urine.
  - 30. A method for detecting *Chlamydia* infection in a biological sample, comprising:
  - (a) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, wherein at least one of the oligonucleotide primers is specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; and
  - (b) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers, thereby detecting *Chlamydia* infection.

- 31. The method of claim 30, wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a polynucleotide sequence of SEO ID NO: 169-174, 181-188, 263, 265 and 267-291.
- 32. A method for detecting *Chlamydia* infection in a biological sample, comprising:
- (a) contacting the sample with one or more oligonucleotide probes specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; and
  - (b) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe, thereby detecting *Chlamydia* infection.
  - 33. The method of claim 32 wherein the probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
  - 34. A method for detecting *Chlamydia* infection in a biological sample, comprising:
  - (a) contacting the biological sample with a binding agent which is capable of binding to a polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291, (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
  - (b) detecting in the sample a polypeptide that binds to the binding agent, thereby detecting *Chlamydia* infection in the biological sample.
  - 35. A method of detecting *Chlamydia* infection in a biological sample, comprising:
  - (a) contacting the biological sample with a binding agent which is capable of binding to a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291, (ii) sequences

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complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and

- (b) detecting in the sample a polypeptide that binds to the binding agent, thereby detecting *Chlamydia* infection in the biological sample.
- 36. The method of any one of claims 34 and 35 wherein the binding agent is a monoclonal antibody.
- 37. The method of any one of claims 34 and 35 wherein the binding agent is a polyclonal antibody.
- 38. The method of any one of claims 34 and 35 wherein the biological sample is selected from the group consisting of whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine.
  - 39. A diagnostic kit comprising:
- (a) a polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291, (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
  - (b) a detection reagent.
  - 40. A diagnostic kit comprising:
- (a) a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
  - (b) a detection reagent.
- 41. The kit of claims 39 or 40 wherein the polypeptide is immobilized on a solid support.

- 42. The kit of claims 39 or 40 wherein the detection reagent comprises a reporter group conjugated to a binding agent.
- 43. The kit of claim 42 wherein the binding agent is selected from the group consisting of anti-immunoglobulins, Protein G, Protein A and lectins.
- 44. The kit of claim 42 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.
  - 45. A diagnostic kit comprising at least two oligonucleotide primers, at least one of the oligonucleotide primers being specific for a polynucleotide molecule comprising a polynucleotide sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
  - 46. A diagnostic kit according to claim 43, wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
  - 47. A diagnostic kit comprising at least one oligonucleotide probe, the oligonucleotide probe being specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
  - 48. A kit according to claim 47, wherein the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
    - 49. A diagnostic kit comprising:
  - (a) at least one antibody, or antigen-binding fragment thereof, according to claim 22; and
    - (b) a detection reagent.
  - 50. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
    - (a) obtaining peripheral blood cells from the patient;

- (b) incubating the cells in the presence of at least one polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions, such that T cells proliferate; and
  - (c) administering to the patient the proliferated T cells.
- 51. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
  - (a) obtaining peripheral blood cells from the patient;
- (b) incubating the cells in the presence of at least one polynucleotide, comprises a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions, such that T cells proliferate; and
  - (c) administering to the patient the proliferated T cells.
- 52. The method of any one of claims 50 and 51 wherein the step of incubating the T cells is repeated one or more times.
- 53. The method of any one of claims 50 and 51 wherein step (a) further comprises separating T cells from the peripheral blood cells, and the cells incubated in step (b) are the T cells.
- 54. The method of any one of claims 50 and 51 wherein step (a) further comprises separating CD4+ cells or CD8+ T cells from the peripheral blood cells, and the cells proliferated in step (b) are CD4+ or CD8+ T cells.
- 55. The method of any one of claims 50 and 51 wherein step (a) further comprises separating gamma/delta T lymphocytes from the peripheral blood cells, and the cells proliferated in step (b) are gamma/delta T lymphocytes.
- 56. The method of any one of claims 50 and 51 wherein step (b) further comprises cloning one or more T cells that proliferated in the presence of the polypeptide.

- 57. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising T cells proliferated in the presence of a polypeptide of claim 1, in combination with a physiologically acceptable carrier.
- 58. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising T cells proliferated in the presence of a polynucleotide of claim 3, in combination with a physiologically acceptable carrier.
- 59. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
- (a) incubating antigen presenting cells in the presence of at least one polypeptide of claim 1;
  - (b) administering to the patient the incubated antigen presenting cells.
- 60. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
- (a) introducing at least one polynucleotide of claim 3 into antigen presenting cells;
  - (b) administering to the patient the antigen presenting cells.
- 61. The method of claims 59 or 60 wherein the antigen presenting cells are selected from the group consisting of dendritic cells. macrophage cells, B cells fibroblast cells, monocyte cells, and stem cells.
- 62. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising antigen presenting cells incubated in the presence of a polypeptide of claim 1, in combination with a physiologically acceptable carrier.
- 63. A pharmaceutical composition for the treatment if *Chlamydia* infection in a patient, comprising antigen presenting cells incubated in the presence of a polynucleotide of claim 3, in combination with a physiologically acceptable carrier.
- 64. A polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said immunogenic portion comprises a sequence of SEQ ID NO: 246, 247 and 254-256.

- 65. An immunogenic epitope of a *Chlamydia* antigen, comprising a sequence of SEQ ID NO: 246, 247 or 254-256.
- 66. An isolated polypeptide comprising a sequence recited in any one of SEQ ID NO: 224-262, 246, 247, 254-256, 292 and 294-305.
- 67. A recombinant fusion polypeptide comprising a an amino acid sequence of a Ra12 polypeptide and an amino acid sequence of a Chlamydial polypeptide.
  - 68. The recombinant polypeptide of claims 67, wherein the Chlamydial polypeptide is a Pmp polypeptide.
  - 69. The recombinant polypeptide of claims 67, wherein the Chlamydial polypeptide is a PmpA, PmpF, PmpH, PmpB, or PmpC.
  - 70. The recombinant polypeptide of claims 67, wherein the amino acid sequence of the fusion polypeptide is a sequence selected from the group consisting of SEQ ID NOs: 309, 313, 317, 321, 325, 329, 333, 337, 341, 345, 349, 353 and 357.
  - 71. A recombinant DNA molecule encoding a fusion polypeptide according to claim 67.

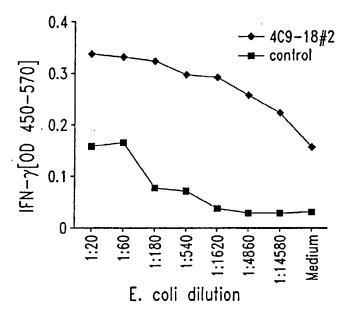


Fig. 1

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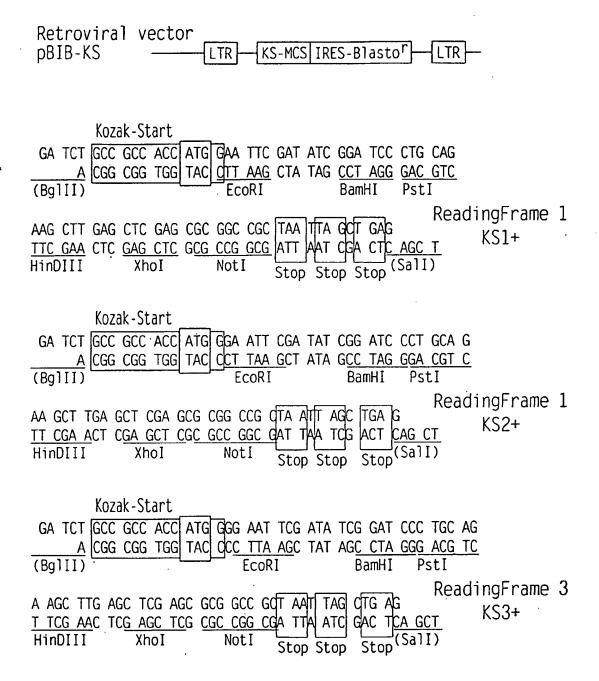


Fig. 2

## Chlamydia C17.8 Peptide Screen

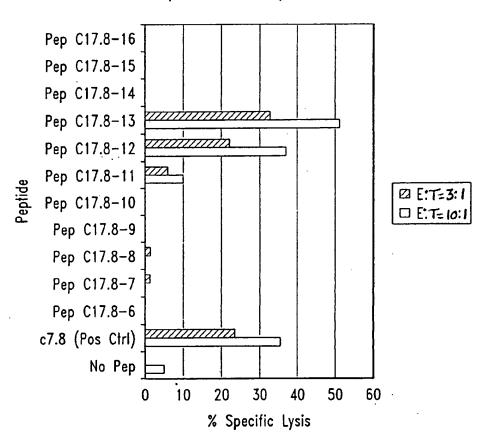
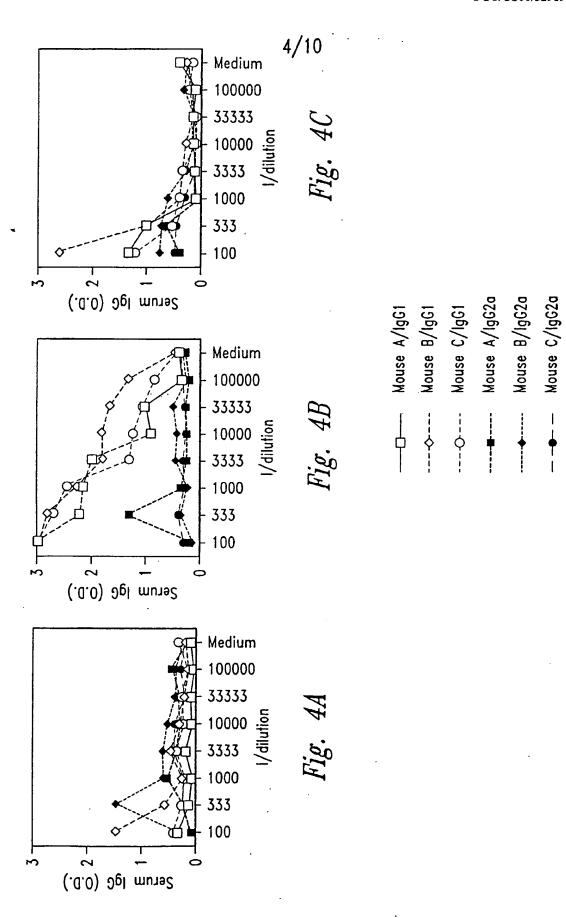
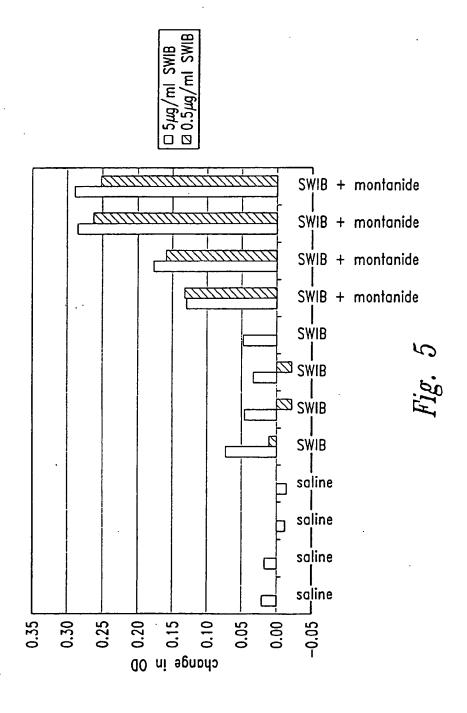


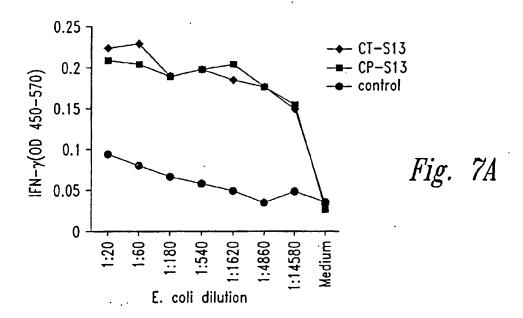
Fig. 3

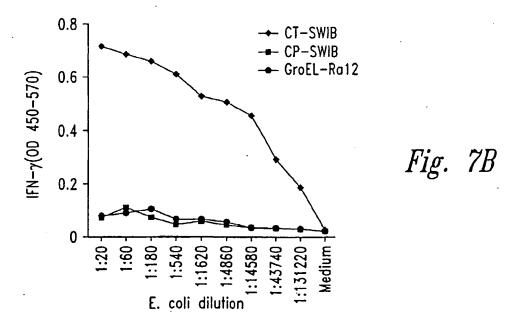




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Fig. 6





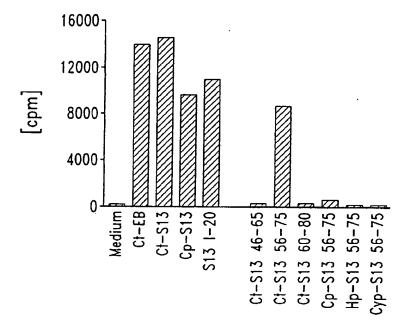
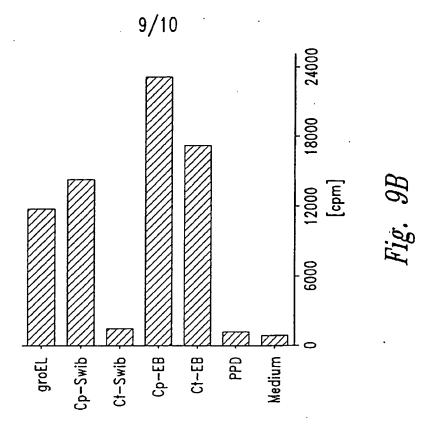
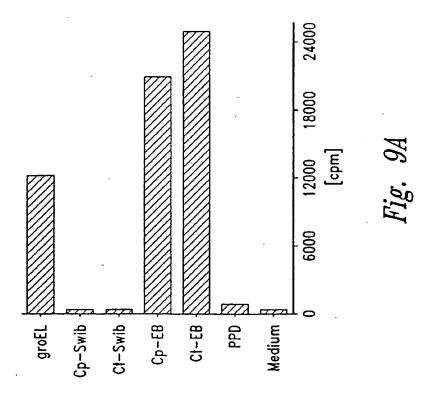


Fig. 8





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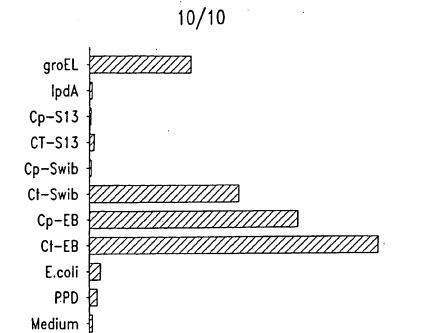
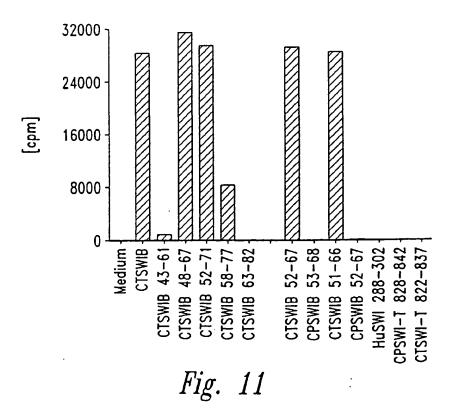


Fig. 10

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<212> PRT

<213> Chlamydia trachomatis

<400> 17

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<211> 18 <212> PRT <213> Chlamydia trachomatis

<210> 19 <211> 18 <212> PRT <213> Chlamydia trachomatis

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Arg Pro

<210> 20 <211> 216 <212> PRT <213> Chlamydia trachomatis

22135 Chiamydia Crachomatis

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<211> 363

7

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Ile Ala Ala Arg Asn Ile Gly Gly His Lys Glu Glu Ile Asp Tyr Ser

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Ala	Val	Pro	Ser 180		Ile	Phe	Thr	Phe 185	Pro		Val	Ala	Ser 190	Val		
Leu	Ser	Pro 195	Thr	Ala	Ala	Gln	Gln 200	His	Leu	Leu	Leu	Arg 205		Leu	Phe	
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Glu 225	Gly	Val	Trp	Lys	Thr 230	Ser										
		210> 211>														
			DNA													
			Chla	amydi	ia pr	neumo	oniae	<b>e</b>		•	•					
		400>														
_	-					_		-	-	_	_				gattta	
															tgggaa gatgcg	120 180
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		210> 211>														
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			Chla	ımydi	a pr	eumo	niae	=								
		100>	-					_								
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aaga													-	_	_	369
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Thr Glu Glu Glu Val Gly Arg Leu Asn Ser Leu Leu Gln Ser Glu Tyr
Thr Val Glu Gly Asp Leu Arg Arg Val Gln Ser Asp Ile Lys Arg
Leu Ile Ala Ile His Ser Tyr Arg Gly Gln Arg His Arg Leu Ser Leu
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<223> Made in the lab

<213> Chlamydia pneumoniae

<400> 32

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<212> DNA

<213> Chlamydia trachomatis

<400> 33

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Leu Phe Val Asn Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr
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Lys Ala Asn Met Gly
    50
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      <211> 55
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      <211> 33
      <212> DNA
      <213> Chlamydia pneumoniae
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cgccgtgggc gatttagcga aaaatgattc ttctattcaa gtacgcatca ctgcttatcg 180
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gcctcatagt ggtgtattaa ctggcataga tcaagcttta atgacctgtg agatgttaaa 360
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<221> unsure
<222> (23)
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<211> 427
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<221> unsure
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tetaageest gacacattet ttgaacaace ttatgeeegt gttegggata agecaactet 180
cgcccccgaa acatacaaga aacctttact ttatttcctt tctcaataaa ggctctagct 240
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<223> n=A,T,C or G
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aaagetttta acaactttcc aatcactaat aaaattcaat gcaacgggtt attcactccc 240
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tcatcaggcg ttcctaattt atgtagtcta agaaccagaa ttattaatac aggattgact 480
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cgtttgatgt gtatactatg tcgtgtaagc ctttttggtt acttctgaca ctagccccca 180
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<400> 50
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* aaaagctctg ggagcatgtt cttagtctca gcagatatta ttgcatcaag aatggaaggc 240
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<400> 66

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<211> 276

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<213> Chlamydia

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<220>
<221> unsure
<222> (34)
<223> n=A,T,C or G
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3031

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Ile Lys Lys His Asn Cys Gln Asp Gln Lys Asn Lys Arg Asn Ile Leu
Pro Asp Ala Asn Leu Ala Lys Val Phe Gly Ser Ser Asp Pro Ile Asp
Met Phe Gln Met Thr Lys Ala Leu Ser Lys His Ile Val Lys
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Gly	Thr 50		Leu	Asn	Arg	Gly 55		Ile	Pro	Ser	<b>Г</b> уз		Leu	Leu	Ala
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His	Val	Glu	Gly	Phe 85	Ser	Ile	Asn	Tyr	Pro 90		Met	Val	Gln	Arg 95	_
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Ser	Val 210	Ile	Glu	Ala	Ser	Ser 215	Gln	Ile	Leu	Ala	Leu 220	Asn	Asn	Pro	Asp
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Gly	Val 290	Ile	Cys	Asp	Glu	Arg 295	Gly	Val	Ile	Pro	Thr 300	Asp	Ala	Thr	Met
Arg 305	Thr	Asn	Val	Pro	Asn 310	Ile	Tyr	Ala	Ile	Gly 315	Asp	Ile	Thr	Gly	Lys 320
Trp	Gln	Leu	Ala	His 325	Val	Ala	Ser	His	Gln 330	Gly	Ile	Ile	Ala	Ala 335	Arg

Asn Ile Gly Gly His Lys Glu Glu Ile Asp Tyr Ser Ala Val Pro Ser 340 345 350

Val Ile Phe Thr Phe Pro Glu Val Ala Ser Val Gly Leu Ser Pro Thr 355 360 365

Ala Ala Gln Gln Lys Ile Pro Val Lys Val Thr Lys Phe Pro Phe 370 375 380

Arg Ala Ile Gly Lys Ala Val Ala Met Gly Glu Ala Asp Gly Phe Ala '385 390 395 400

Ala Ile Ile Ser His Glu Thr Thr Gln Gln Ile Leu Gly Ala Tyr Val 405 410 415

Ile Gly Pro His Ala Ser Ser Leu Ile Ser Glu Ile Thr Leu Ala Val 420 425 430

Arg Asn Glu Leu Thr Leu Pro Cys Ile Tyr Glu Thr Ile His Ala His 435 440 445

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Glu Ala Arg Ala Ser Glu Leu Thr Glu Glu Glu Val Gly Arg Leu Asn 50 55 60

Ser Leu Leu Gln Ser Glu Tyr Thr Val Glu Gly Asp Leu Arg Arg Arg 65 70 75 80

Val Gln Ser Asp Ile Lys Arg Leu Ile Ala Ile His Ser Tyr Arg Gly

Gln Arg His Arg Leu Ser Leu Pro Val Arg Gly Gln Arg Thr Lys Thr 100 105 110

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Lys

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Lys Asp Phe Thr Tyr Val Cys Pro Thr Glu Leu His Ala Phe Gln Asp 50 55 60

Arg Leu Val Asp Phe Glu Glu His Gly Ala Val Val Leu Gly Cys Ser 65 70 75 80

Val Asp Asp Ile Glu Thr His Ser Arg Trp Leu Thr Val Ala Arg Asp 85 90 95

Ala Gly Gly Ile Glu Gly Thr Glu Tyr Pro Leu Leu Ala Asp Pro Ser 100 105 110

Phe Lys Ile Ser Glu Ala Phe Gly Val Leu Asn Pro Glu Gly Ser Leu 115 120 125

Ala Leu Arg Ala Thr Phe Leu Ile Asp Lys His Gly Val Ile Arg His 130 135 140

Ala Val Ile Asn Asp Leu Pro Leu Gly Arg Ser Ile Asp Glu Glu Leu 145 150 155 160

Arg Ile Leu Asp Ser Leu Ile Phe Phe Glu Asn His Gly Met Val Cys
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PCT/US00/32919 WO 01/40474 37

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Simple   S															
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PCT/US00/32919 WO 01/40474

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215

230

225

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220

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                                25
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Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr
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His Lys Arg Arg Ala Ala Ala Val Cys Ser Ile Ile Gly Gly Ile
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Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn

Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln
100 105 110
Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser

85

145 150 155 160

Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met

45

165 170 Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val 185 Val Gly Ala Gly Leu Ala Ile Ser Ala Glu Arg Ala Asp Cys Glu Ala 200 205 Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Val Pro Gly 210 215 Glu Glu Asn Ala Cys Glu Lys Lys Val Ala Gly Glu Lys Ala Lys Thr 225 230 235 Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu 250 245 Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met 265 Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile 280 Ile Gly Leu Cys Thr Phe Cys Ala Arg Ala <210> 128 <211> 897 <212> DNA <213> Chlamydia <400> 128 atggetteta tatgtggaeg tttagggtet ggtaeaggga atgetetaaa agettttttt 60 acacageeca geaataaaat ggeaagggta gtaaataaga egaagggaat ggataagaet 120 gttaaggtcg ccaagtctgc tgccgaattg accgcaaata ttttggaaca agctggaggc 180 gegggetett cegeacacat tacagettee caagtgteea aaggattagg ggataegaga 240 actgttgtcg ctttagggaa tgcctttaac ggagcgttgc caggaacagt tcaaagtgcg 300 caaagettet teteteacat gaaagetget agteagaaaa egeaagaagg ggatgagggg 360 etcacageag atetttgtgt gtetcataag egeagagegg etgeggetgt etgtggette 420 ateggaggaa ttacctacct cgcgacattc ggagttatcc gtccgattct gtttgtcaac 480 aaaatgctgg tgaacccgtt tetttettee caaactaaag caaatatggg atettetgtt 540 agetatatta tggeggetaa ceatgeageg tetgtggtgg gtgetggaet egetateagt 600 geggaaagag cagattgega agceegetge getegtattg egagagaaga gtegttaete 660 gaagtqtcgg gagaggaaaa tgcttgcgag aagagagtcg ctggagagaa agccaagacg 720 ttcacgcgca tcaagtatgc actcctcact atgctcgaga agtttttgga atgcgttgcc 780 gacgttttca aattggtgcc gctgcctatt acaatgggta ttcgtgcgat tgtggctgct. 840 ggatgtacgt tcacttctgc aattattgga ttgtgcactt tctgcgccag agcataa 897 <210> 129 <211> 298 <212> PRT <213> Chlamydia <400> 129 Met Ala Ser Ile Cys Gly Arg Leu Gly Ser Gly Thr Gly Asn Ala Leu Lys Ala Phe Phe Thr Gln Pro Ser Asn Lys Met Ala Arg Val Val Asn Lys Thr Lys Gly Met Asp Lys Thr Val Lys Val Ala Lys Ser Ala Ala 40 Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser 55 60 Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Thr Arg 70 75 Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr

Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln 105 Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser 125 120 His Lys Arg Arg Ala Ala Ala Val Cys Gly Phe Ile Gly Gly Ile 140 135 Thr Tyr Leu Ala Thr Phe Gly Val Ile Arg Pro Ile Leu Phe Val Asn 155 150 Lys Met Leu Val Asn Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met 170 175 Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val 185 190 Val Gly Ala Gly Leu Ala Ile Ser Ala Glu Arg Ala Asp Cys Glu Ala 200 205 Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Val Ser Gly 215 220 Glu Glu Asn Ala Cys Glu Lys Arg Val Ala Gly Glu Lys Ala Lys Thr 230 235 Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu 245 250 Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met 265 270 Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile 280 285 Ile Gly Leu Cys Thr Phe Cys Ala Arg Ala 290 295 <210> 130 <211> 897

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480

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540

600

660

720

780

840

897

WO 01/40474 PCT/US00/32919

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				aagcacttgg		240
				cagcaaccat		300
				aagaaacatg		360.
				aggcttgtaa		420
				cattactcgt		480
				tgggagcttc		540
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Met Ala Ser Val Cys Gly Arg Leu Ser Ala Gly Val Gly Asn Arg Phe

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1				5					10					15	
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Ile Gly Ile Thr Phe Glu Lys Lys Ser Gln Lys Thr Arg Thr Tyr Tyr 790 795 Tyr Phe Leu Gly Ala Tyr Ile Gln Asp Leu Lys Arg Asp Val Glu Ser 805 810 Gly Pro Val Val Leu Leu Lys Asn Ala Val Ser Trp Asp Ala Pro Met 820 825 Ala Asn Leu Asp Ser Arg Ala Tyr Met Phe Arg Leu Thr Asn Gln Arg 840 Ala Leu His Arg Leu Gln Thr Leu Leu Asn Val Ser Cys Val Leu Arg 855 860 Gly Gln Ser His Ser Tyr Ser Leu Asp Leu Gly Thr Thr Tyr Arg Phe 870 875 <210> 176 <211> 982 <212> PRT <213> Chlamydia <220> <221> VARIANT <222> (1)...(982) <223> Xaa = Any Amino Acid <400> 176 Met Ile Pro Gln Gly Ile Tyr Asp Gly Glu Thr Leu Thr Val Ser Phe 10 Pro Tyr Thr Val Ile Gly Asp Pro Ser Gly Thr Thr Val Phe Ser Ala 20 25 Gly Glu Leu Thr Leu Lys Asn Leu Asp Asn Ser Ile Ala Ala Leu Pro 40 Leu Ser Cys Phe Gly Asn Leu Leu Gly Ser Phe Thr Val Leu Gly Arg 55 60 Gly His Ser Leu Thr Phe Glu Asn Ile Arg Thr Ser Thr Asn Gly Ala 70 75 Ala Leu Ser Asn Ser Ala Ala Asp Gly Leu Phe Thr Ile Glu Gly Phe 85 90 Lys Glu Leu Ser Phe Ser Asn Cys Asn Ser Leu Leu Ala Val Leu Pro 105 100 Ala Ala Thr Thr Asn Lys Gly Ser Gln Thr Pro Thr Thr Thr Ser Thr 120 125 Pro Ser Asn Gly Thr Ile Tyr Ser Lys Thr Asp Leu Leu Leu Leu Asn 135 140 Asn Glu Lys Phe Ser Phe Tyr Ser Asn Leu Val Ser Gly Asp Gly Gly 150 155 Ala Ile Asp Ala Lys Ser Leu Thr Val Gln Gly Ile Ser Lys Leu Cys 170 165 Val Phe Gln Glu Asn Thr Ala Gln Ala Asp Gly Gly Ala Cys Gln Val 180 185 Val Thr Ser Phe Ser Ala Met Ala Asn Glu Ala Pro Ile Ala Phe Val 200 Ala Asn Val Ala Gly Val Arg Gly Gly Gly Ile Ala Ala Val Gln Asp 215 220 Gly Gln Gln Gly Val Ser Ser Ser Thr Ser Thr Glu Asp Pro Val Val 230 235 Ser Phe Ser Arg Asn Thr Ala Val Glu Phe Asp Gly Asn Val Ala Arg 250 Val Gly Gly Gle Tyr Ser Tyr Gly Asn Val Ala Phe Leu Asn Asn

265

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		_		Asn	405					410					415	
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69

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Leu Cys Gly Ser Tyr Leu Phe Gly Asp Ala Phe Ile Arg Ala Ser Tyr
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Arg Pro Phe Val Gln Ala Glu Phe Ser Tyr Ala Asp His Glu Ser Phe
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His Pro Asn Lys Tyr Ser Phe Met Ala Ala Tyr Ile Cys Asp Ala Tyr
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	Phe 225		Ser	Asn	Asn	Cys 230		Leu	Phe	Phe	Ile 235		Asn	Ala	Cys	Cys 240
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	465					470					475					Gly 480
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			595	-			_	Leu 600					605			
	GTÅ	ьеи	Trp	Thr	LIP	GIA	ırp	Ala	гÀЗ	Inr	GIN	Asp	PTO	GIU	PEO	ATG

610 615 620 Ser Ser Ala Thr Ile Thr Asp Pro Gln Lys Ala Asn Arg Phe His Arg 630 635 640 Thr Leu Leu Leu Thr Trp Leu Pro Ala Gly Tyr Val Pro Ser Pro Lys 645 650 His Arg Ser Pro Leu Ile Ala Asn Thr Leu Trp Gly Asn Met Leu Leu 665 Ala Thr Glu Ser Leu Lys Asn Ser Ala Glu Leu Thr Pro Ser Gly His 675 680 685 Pro Phe Trp Gly Ile Thr Gly Gly Gly Leu Gly Met Met Val Tyr Gln 695 700 Asp Pro Arg Glu Asn His Pro Gly Phe His Met Arg Ser Ser Gly Tyr 710 715 Ser Ala Gly Met Ile Ala Gly Gln Thr His Thr Phe Ser Leu Lys Phe 725 730 735 Ser Gln Thr Tyr Thr Lys Leu Asn Glu Arg Tyr Ala Lys Asn Asn Val 745 Ser Ser Lys Asn Tyr Ser Cys Gln Gly Glu Met Leu Phe Ser Leu Gln 755 760 Glu Gly Phe Leu Leu Thr Lys Leu Val Gly Leu Tyr Ser Tyr Gly Asp 770 775 780 His Asn Cys His His Phe Tyr Thr Gln Gly Glu Asn Leu Thr Ser Gln 795 800 790 Gly Thr Phe Arg Ser Gln Thr Met Gly Gly Ala Val Phe Phe Asp Leu 810 815 805 Pro Met Lys Pro Phe Gly Ser Thr His Ile Leu Thr Ala Pro Phe Leu 820 825 830 Gly Ala Leu Gly Ile Tyr Ser Ser Leu Ser His Phe Thr Glu Val Gly 835 840 845 Ala Tyr Pro Arg Ser Phe Ser Thr Lys Thr Pro Leu Ile Asn Val Leu 855 860 Val Pro Ile Gly Val Lys Gly Ser Phe Met Asn Ala Thr His Arg Pro 870 875 Gln Ala Trp Thr Val Glu Leu Ala Tyr Gln Pro Val Leu Tyr Arg Gln 885 890 Glu Pro Gly Ile Ala Thr Gln Leu Leu Ala Ser Lys Gly Ile Trp Phe 900 905 Gly Ser Gly Ser Pro Ser Ser Arg His Ala Met Ser Tyr Lys Ile Ser 915 920 925 Gln Gln Thr Gln Pro Leu Ser Trp Leu Thr Leu His Phe Gln Tyr His 930 935 940 Gly Phe Tyr Ser Ser Ser Thr Phe Cys Asn Tyr Leu Asn Gly Glu Ile 950 955 Ala Leu Arg Phe

<210> 178

<211> 1530

<212> PRT

<213> Chlamydia

Met Ser Ser Glu Lys Asp Ile Lys Ser Thr Cys Ser Lys Phe Ser Leu Ser Val Val Ala Ala Ile Leu Ala Ser Val Ser Gly Leu Ala Ser Cys 25 Val Asp Leu His Ala Gly Gly Gln Ser Val Asn Glu Leu Val Tyr Val

	Gly	Pro 50	Gln	Ala	Val	Leu	Leu 55	Leu	Asp	Gln	Ile	Arg 60	Asp	Leu	Phe	Val
	Gly 65	Ser	Lys	Asp	Ser	Gln 70	Ala	Glu	Gly	Gln	Tyr 75	Arg	Leu	Ile	Val	Gly 80
	Asp	Pro	Ser	Ser	Phe 85	Gln	Glu	Lys	Asp	Ala 90	Asp	Thr	Leu	Pro	Gly 95	Lys
				100		Leu			105					110		
			115			Asp		120					125			
•		130				Leu	135					140				
	145					Gly 150					155					160
					165	Leu				170					175	
				180		Ile			185					190		
			195		_	Gly Gln		200					205			
	_	210		_			215	_		-		220				
	225					Asn 230					235					240
				_	245	Leu		_		250					255	
				260		Ala			265					270		
			275			Ala		280					285			_
		290				Asn	295	_	_			300				
	305			_		Ala 310					315					320
		_			325	Val		_		330					335	
	_	_	_	340		Gly	_	_	345					350		
			355	_		His	_	360		_		-	365			
		370	_	_		Ile	375	-	_		_	380			_	
	385					Phe 390					395					400
					405	Glu				410					415	_
				420		Gly			425		_			430		
			435			Ala		440					445			
		450				Gly	455					460				
	465		_			Gln 470					475	_	_			480
					485	Leu				490					495	
	Asp	Asn	Ile	Val 500	Lys	Thr	Phe	Ala	Ser 505	Asn	Gly	Lys	Ile	Leu 510	Gly	Gly

Gly Ala Ile Leu Ala Thr Gly Lys Val Glu Ile Thr Asn Asn Ser Gly Gly Ile Ser Phe Thr Gly Asn Ala Arg Ala Pro Gln Ala Leu Pro Thr Gln Glu Glu Phe Pro Leu Phe Ser Lys Lys Glu Gly Arg Pro Leu Ser Ser Gly Tyr Ser Gly Gly Gly Ala Ile Leu Gly Arg Glu Val Ala Ile Leu His Asn Ala Ala Val Val Phe Glu Gln Asn Arg Leu Gln Cys Ser Glu Glu Glu Ala Thr Leu Leu Gly Cys Cys Gly Gly Gly Ala Val His Gly Met Asp Ser Thr Ser Ile Val Gly Asn Ser Ser Val Arg Phe Gly Asn Asn Tyr Ala Met Gly Gln Gly Val Ser Gly Gly Ala Leu Leu Ser Lys Thr Val Gln Leu Ala Gly Asn Gly Ser Val Asp Phe Ser Arg Asn Ile Ala Ser Leu Gly Gly Gly Ala Leu Gln Ala Ser Glu Gly Asn Cys Glu Leu Val Asp Asn Gly Tyr Val Leu Phe Arg Asp Asn Arg Gly Arg Val Tyr Gly Gly Ala Ile Ser Cys Leu Arg Gly Asp Val Val Ile Ser Gly Asn Lys Gly Arg Val Glu Phe Lys Asp Asn Ile Ala Thr Arg Leu Tyr Val Glu Glu Thr Val Glu Lys Val Glu Glu Val Glu Pro Ala Pro Glu Gln Lys Asp Asn Asn Glu Leu Ser Phe Leu Gly Ser Val Glu Gln Ser Phe Ile Thr Ala Ala Asn Gln Ala Leu Phe Ala Ser Glu Asp Gly Asp Leu Ser Pro Glu Ser Ser Ile Ser Ser Glu Glu Leu Ala Lys Arg Arg Glu Cys Ala Gly Gly Ala Ile Phe Ala Lys Arg Val Arg Ile Val Asp Asn Gln Glu Ala Val Val Phe Ser Asn Asn Phe Ser Asp Ile Tyr Gly Gly Ala Ile Phe Thr Gly Ser Leu Arg Glu Glu Asp Lys Leu Asp Gly Gln Ile Pro Glu Val Leu Ile Ser Gly Asn Ala Gly Asp Val Val Phe Ser Gly Asn Ser Ser Lys Arg Asp Glu His Leu Pro His Thr Gly Gly Gly Ala Ile Cys Thr Gln Asn Leu Thr Ile Ser Gln Asn Thr Gly Asn Val Leu Phe Tyr Asn Asn Val Ala Cys Ser Gly Gly Ala Val Arg Ile Glu Asp His Gly Asn Val Leu Leu Glu Ala Phe Gly Gly Asp Ile Val Phe Lys Gly Asn Ser Ser Phe Arg Ala Gln Gly Ser Asp Ala Ile Tyr Phe Ala Gly Lys Glu Ser His Ile Thr Ala Leu Asn Ala Thr Glu Gly His Ala Ile Val Phe His Asp Ala Leu Val Phe Glu Asn Leu Lys Glu Arg Lys Ser Ala Glu Val Leu Leu Ile Asn Ser Arg Glu Asn Pro 

74

Gly Tyr Thr Gly Ser Ile Arg Phe Leu Glu Ala Glu Ser Lys Val Pro 985 Gln Cys Ile His Val Gln Gln Gly Ser Leu Glu Leu Leu Asn Gly Ala 1000 1005 Thr Leu Cys Ser Tyr Gly Phe Lys Gln Asp Ala Gly Ala Lys Leu Val 1010 1015 1020 Leu Ala Ala Gly Ser Lys Leu Lys Ile Leu Asp Ser Gly Thr Pro Val 1030 1035 Gln Gly His Ala Ile Ser Lys Pro Glu Ala Glu Ile Glu Ser Ser 1045 1050 1055 Glu Pro Glu Gly Ala His Ser Leu Trp Ile Ala Lys Asn Ala Gln Thr 1060 1065 1070 Thr Val Pro Met Val Asp Ile His Thr Ile Ser Val Asp Leu Ala Ser 1085 1080 Phe Ser Ser Gln Gln Glu Gly Thr Val Glu Ala Pro Gln Val Ile 1090 1095 Val Pro Gly Gly Ser Tyr Val Arg Ser Gly Glu Leu Asn Leu Glu Leu 1105 1110 1115 1120 Val Asn Thr Thr Gly Thr Gly Tyr Glu Asn His Ala Leu Leu Lys Asn 1125 1130 1135 Glu Ala Lys Val Pro Leu Met Ser Phe Val Ala Ser Ser Asp Glu Ala 1140 1145 1150 Ser Ala Glu Ile Ser Asn Leu Ser Val Ser Asp Leu Gln Ile His Val 1160 1165 1155 Ala Thr Pro Glu Ile Glu Glu Asp Thr Tyr Gly His Met Gly Asp Trp 1175 1180 Ser Glu Ala Lys Ile Gln Asp Gly Thr Leu Val Ile Asn Trp Asn Pro 1190 1195 1200 Thr Gly Tyr Arg Leu Asp Pro Gln Lys Ala Gly Ala Leu Val Phe Asn 1205 1210 1215 Ala Leu Trp Glu Glu Gly Ala Val Leu Ser Ala Leu Lys Asn Ala Arg 1220 1225 1230 Phe Ala His Asn Leu Thr Ala Gln Arg Met Glu Phe Asp Tyr Ser Thr 1240 1245 Asn Val Trp Gly Phe Ala Phe Gly Gly Phe Arg Thr Leu Ser Ala Glu 1250 1255 1260 Asn Leu Val Ala Ile Asp Gly Tyr Lys Gly Ala Tyr Gly Gly Ala Ser 1270 1275 Ala Gly Val Asp Ile Gln Leu Met Glu Asp Phe Val Leu Gly Val Ser 1285 1290 1295 Gly Ala Ala Phe Leu Gly Lys Met Asp Ser Gln Lys Phe Asp Ala Glu 1300 1305 1310 Val Ser Arg Lys Gly Val Val Gly Ser Val Tyr Thr Gly Phe Leu Ala 1320 1325 Gly Ser Trp Phe Phe Lys Gly Gln Tyr Ser Leu Gly Glu Thr Gln Asn 1330 1335 1340 Asp Met Lys Thr Arg Tyr Gly Val Leu Gly Glu Ser Ser Ala Ser Trp 1350 1355 1360 Thr Ser Arg Gly Val Leu Ala Asp Ala Leu Val Glu Tyr Arg Ser Leu 1365 1370 1375 Val Gly Pro Val Arg Pro Thr Phe Tyr Ala Leu His Phe Asn Pro Tyr 1380 1385 1390 Val Glu Val Ser Tyr Ala Ser Met Lys Phe Pro Gly Phe Thr Glu Gln 1400 1405 1395 Gly Arg Glu Ala Arg Ser Phe Glu Asp Ala Ser Leu Thr Asn Ile Thr 1410 1415 1420 Ile Pro Leu Gly Met Lys Phe Glu Leu Ala Phe Ile Lys Gly Gln Phe 1425 1430 1435

Ser Glu Val Asn Ser Leu Gly Ile Ser Tyr Ala Trp Glu Ala Tyr Arg 1450 1445 Lys Val Glu Gly Gly Ala Val Gln Leu Leu Glu Ala Gly Phe Asp Trp 1465 1460 Glu Gly Ala Pro Met Asp Leu Pro Arg Gln Glu Leu Arg Val Ala Leu 1480 1475 1485 Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe Ser Thr Val Leu Gly Leu 1495 1500 Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr Asp Ser Lys Leu Gly Tyr 1505 1510 1515 Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe 1525

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<211> 1776

<212> PRT

<213> Chlamydia

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Thr Glu Gln Thr Lys Ser Asn Gly Asn Gln Asp Gly Ser Ser Glu Thr

Lys Asp Thr Gln Val Ser Glu Ser Pro Glu Ser Thr Pro Ser Pro Asp

300

295

	305					310					315					320
	Asp	Val	Leu	Gly	Lys 325	Gly	Gly	Gly	Ile	Tyr 330	Thr	Glu	Lys	Ser	Leu 335	Thr
	Ile	Thr	Gly	Ile 340	Thr	Gly	Thr	Ile	Asp 345	Phe	Val	Ser	Asn	Ile 350	Ala	Thr
	Asp	Ser	Gly 355	Ala	Gly	Val	Phe	Thr 360	Lys	Glu	Asn	Leu	Ser 365	CAa	Thr	Asn
	Thr	Asn 370	Ser	Leu	Gln	Phe	Leu 375	Lys	Asn	Ser	Ala	Gly 380	Gln	His	Gly	Gly
	Gly 385	Ala	Tyr	Val	Thr	Gln 390	Thr	Met	Ser	Val	Thr 395	Asn	Thr	Thr	Ser	Glu 400
•					405	Pro			_	410					415	
			-	420		Gly			425					430		
			435	•		Val		440		_			445			
		450				Thr	455					460			_	
	465					Pro 470					475					480
					485	Lys Leu			_	490					495	
				500		Thr			505					510	_	
			515		_	Ile		520					525			
		530				Lys	535					540	_	_	_	_
	545		-		-	550 Ser		_			555					560
			_		565	Asp		_		570	٠.	-		=	<b>575</b>	
	Ser	Ala	Ala	580 Lys	Glu	Gly	Gly	Val	585 Ile	His	Ser	Lys	Thr	590 Val	Thr	Leu
	Ser	Asn	595 Leu	Lys	Ser	Thr	Phe	600 Thr	Phe	Ala	Asp	Asn	605 Thr	Val	Lys	Ala
		610 Val	Glu	Ser	Thr	Pro	615 Glu	Ala	Pro	Glu		620 Ile	Pro	Pro	Val	
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	Ser	Ser	Ala		645 Thr	Asn	Leu	Glu		650 Ser	Gln	Gly	Asp		655 Ala	Asp
	Thr	Gly	Thr 675	660 Gly	Val	Val	Asn	Asn 680	665 Glu	Ser	Gln	Asp		670 Ser	Asp	Thr
	Gly	Asn 690		Glu	Ser	Gly	Glu 695		Leu	Gln	Asp	Ser 700	685 Thr	Gln	Ser	Asn
	Glu 705		Asn	Thr	Leu	Pro 710		Ser	Ser	Ile	Asp 715		Ser	Asn	Glu	Asn 720
		Asp	Glu	Ser	Ser 725	Asp	Ser	His	Thr	Glu 730		Ile	Thr	Asp	Glu 735	
	Val	Ser	Ser	Ser 740		Lys	Ser	Gly	Ser 745	-	Thr	Pro	Gln	Asp 750		Gly
	Ala	Ala	Ser 755	Ser	Gly	Ala	Pro	Ser 760	Gly	Asp	Gln	Ser	Ile 765		Ala	Asn
	Ala	Cys	Leu	Ala	Lys	Ser	Tyr	Ala	Ala	Ser	Thr	Asp	Ser	Ser	Pro	Val

		770					775					780				
	Ser 785	Asn	Ser	Ser	Gly	Ser 790	Asp	Val	Thr	Ala	Ser 795	Ser	Asp	Asn	Pro	Asp 800
					805				_	810					815	Glu
	Pro	Glu	Ala	Gly 820	Ser	Thr	Thr	Glu	Thr 825	Pro	Thr	Leu	Ile	Gly 830	Gly	Gly
			835	Gly				840					845	_		_
		850		Gly			855		_			860		_		
•	865	_		Asn		870		_			875		_			880
			_	Ser	885			_	_	890					895	
				Ser 900					905				_	910		
	-		915	Thr				920					925		-	
		930		Asn			935				_	940		_	_	_
	945		_	Gly Phe		950					955					960
	_			Asp	965					970					975	_
				980 Phe					985					990		
			995			_		1000	כ		_	_	1005	5		_
		1010	)	Val			101	5	_			1020	)			
	1025	5		Glu		1030					1035	5				1040
				Leu	1045	5				1050	)				1055	5
				Thr 1060	)			_	1069	5				1070	)	-
			1075		_			1080	)	_			1085	5		
		1090	)	Thr	_		1095	5		_		1100	)			
	1105	_	Cys	Phe	_	1110		GIU	IAT		1115		ser	Ser		1120
			Ser	Thr		Cys		Ile	Ala		Asp		Lys	Leu		Met
	Gln	Ala	Ala	Lys 1140	Gly		Thr	Ile	Ser 1149	Phe		Asp	Ala	Ile 1150	Arg	
	Ser	Thr	Lys 1155	Lys	Thr	Gly	Thr	Gln 1160		Thr	Ala	Tyr	Asp 1165		Leu	Asp
	Ile	Asn 1170	_	Ser	Glu	Asp	Ser 1175	Glu		Val	Asn	Ser 1180		Phe	Thr	Gly
	Thr 1185		Leu	Phe	Ser	Ser 1190		Leu	His	Glu	Asn 1195	_	Ser	Tyr	Ile	Pro 1200
			Val	Val	Leu 1205		Ser	Gly	Ser	Leu 1210		Leu	Lys	Pro	Asn 1215	
	Glu	Leu	His	Val 1220		Ser	Phe	Glu	Gln 1225	_	Glu	Gly	Ser	Ser 1230		Val
	Met	Thr	Pro	Gly	Ser	Val	Leu	Ser	Asn	Gln	Thr	Val	Ala	Asp	Gly	Ala

1235 1240 1245 Leu Val Ile Asn Asn Met Thr Ile Asp Leu Ser Ser Val Glu Lys Asn 1250 1255 1260 Gly Ile Ala Glu Gly Asn Ile Phe Thr Pro Pro Glu Leu Arg Ile Ile 1270 1275 1280 Asp Thr Thr Ser Gly Ser Gly Gly Thr Pro Ser Thr Asp Ser Glu 1285 1290 1295 Ser Asn Gln Asn Ser Asp Asp Thr Lys Glu Gln Asn Asn Asn Asp Ala 1300 1305 1310 Ser Asn Gln Gly Glu Ser Ala Asn Gly Ser Ser Ser Pro Ala Val Ala 1315 1320 1325 "Ala Ala His Thr Ser Arg Thr Arg Asn Phe Ala Ala Ala Ala Thr Ala 1330 1335 1340 Thr Pro Thr Thr Pro Thr Ala Thr Thr Thr Ser Asn Gln Val 1350 1355 1360 Ile Leu Gly Gly Glu Ile Lys Leu Ile Asp Pro Asn Gly Thr Phe Phe 1365 1370 1375 Gln Asn Pro Ala Leu Arg Ser Asp Gln Gln Ile Ser Leu Leu Val Leu 1380 1385 1390 Pro Thr Asp Ser Ser Lys Met Gln Ala Gln Lys Ile Val Leu Thr Gly 1395 1400 1405 Asp Ile Ala Pro Gln Lys Gly Tyr Thr Gly Thr Leu Thr Leu Asp Pro 1410 1415 1420 Asp Gln Leu Gln Asn Gly Thr Ile Ser Ala Leu Trp Lys Phe Asp Ser 1425 1430 1435 Tyr Arg Gln Trp Ala Tyr Val Pro Arg Asp Asn His Phe Tyr Ala Asn 1445 1450 1455 Ser Ile Leu Gly Ser Gln Met Ser Met Val Thr Val Lys Gln Gly Leu 1460 1465 Leu Asn Asp Lys Met Asn Leu Ala Arg Phe Asp Glu Val Ser Tyr Asn 1475 1480 1485 Asn Leu Trp Ile Ser Gly Leu Gly Thr Met Leu Ser Gln Val Gly Thr 1495 1500 Pro Thr Ser Glu Glu Phe Thr Tyr Tyr Ser Arg Gly Ala Ser Val Ala 1510 1515 Leu Asp Ala Lys Pro Ala His Asp Val Ile Val Gly Ala Ala Phe Ser 1525 1530 Lys Met Ile Gly Lys Thr Lys Ser Leu Lys Arg Glu Asn Asn Tyr Thr 1540 1545 1550 His Lys Gly Ser Glu Tyr Ser Tyr Gln Ala Ser Val Tyr Gly Gly Lys 1555 · 1560 1565 Pro Phe His Phe Val Ile Asn Lys Lys Thr Glu Lys Ser Leu Pro Leu 1575 1580 Leu Leu Gln Gly Val Ile Ser Tyr Gly Tyr Ile Lys His Asp Thr Val 1590 1595 1600 Thr His Tyr Pro Thr Ile Arg Glu Arg Asn Gln Gly Glu Trp Glu Asp 1605 1610 Leu Gly Trp Leu Thr Ala Leu Arg Val Ser Ser Val Leu Arg Thr Pro 1620 1625 1630 Ala Gln Gly Asp Thr Lys Arg Ile Thr Val Tyr Gly Glu Leu Glu Tyr 1640 1645 Ser Ser Ile Arg Gln Lys Gln Phe Thr Glu Thr Glu Tyr Asp Pro Arg 1655 Tyr Phe Asp Asn Cys Thr Tyr Arg Asn Leu Ala Ile Pro Met Gly Leu 1670 1675 Ala Phe Glu Gly Glu Leu Ser Gly Asn Asp Ile Leu Met Tyr Asn Arg 1685 1690 Phe Ser Val Ala Tyr Met Pro Ser Ile Tyr Arg Asn Ser Pro Thr Cys

1705 1700 1710 Lys Tyr Gln Val Leu Ser Ser Gly Glu Gly Glu Ile Ile Cys Gly 1715 1720 1725 Val Pro Thr Arg Asn Ser Ala Arg Gly Glu Tyr Ser Thr Gln Leu Tyr 1730 1735 1740 Pro Gly Pro Leu Trp Thr Leu Tyr Gly Ser Tyr Thr Ile Glu Ala Asp 1745 1750 1755 1760 Ala His Thr Leu Ala His Met Met Asn Cys Gly Ala Arg Met Thr Phe 1770 <210> 180 **~** <211> 1752 <212> PRT <213> Chlamydia <400> 180 Met Lys Trp Leu Ser Ala Thr Ala Val Phe Ala Ala Val Leu Pro Ser 10 Val Ser Gly Phe Cys Phe Pro Glu Pro Lys Glu Leu Asn Phe Ser Arg 20 25 Val Glu Thr Ser Ser Ser Thr Thr Phe Thr Glu Thr Ile Gly Glu Ala 40 Gly Ala Glu Tyr Ile Val Ser Gly Asn Ala Ser Phe Thr Lys Phe Thr 55 60 Asn Ile Pro Thr Thr Asp Thr Thr Thr Pro Thr Asn Ser Asn Ser Ser 70 75 Ser Ser Ser Gly Glu Thr Ala Ser Val Ser Glu Asp Ser Asp Ser Thr 85 90 Thr Thr Thr Pro Asp Pro Lys Gly Gly Gly Ala Phe Tyr Asn Ala His 105 Ser Gly Val Leu Ser Phe Met Thr Arg Ser Gly Thr Glu Gly Ser Leu 120 125 Thr Leu Ser Glu Ile Lys Met Thr Gly Glu Gly Gly Ala Ile Phe Ser 135 140 Gln Gly Glu Leu Leu Phe Thr Asp Leu Thr Ser Leu Thr Ile Gln Asn 150 155 Asn Leu Ser Gln Leu Ser Gly Gly Ala Ile Phe Gly Gly Ser Thr Ile 165 170 Ser Leu Ser Gly Ile Thr Lys Ala Thr Phe Ser Cys Asn Ser Ala Glu 180 185 Val Pro Ala Pro Val Lys Lys Pro Thr Glu Pro Lys Ala Gln Thr Ala 200 Ser Glu Thr Ser Gly Ser Ser Ser Ser Gly Asn Asp Ser Val Ser 215 220 Ser Pro Ser Ser Ser Arg Ala Glu Pro Ala Ala Asn Leu Gln Ser 230 235 His Phe Ile Cys Ala Thr Ala Thr Pro Ala Ala Gln Thr Asp Thr Glu 245 250 Thr Ser Thr Pro Ser His Lys Pro Gly Ser Gly Gly Ala Ile Tyr Ala 265 Lys Gly Asp Leu Thr Ile Ala Asp Ser Gln Glu Val Leu Phe Ser Ile 275 280 Asn Lys Ala Thr Lys Asp Gly Gly Ala Ile Phe Ala Glu Lys Asp Val 295 300 Ser Phe Glu Asn Ile Thr Ser Leu Lys Val Gln Thr Asn Gly Ala Glu 310 315 Glu Lys Gly Gly Ala Ile Tyr Ala Lys Gly Asp Leu Ser Ile Gln Ser

330

WO 01/40474

80

PCT/US00/32919

Ser Lys Gln Ser Leu Phe Asn Ser Asn Tyr Ser Lys Gln Gly Gly Gly 345 Ala Leu Tyr Val Glu Gly Gly Ile Asn Phe Gln Asp Leu Glu Glu Ile 360 Arg Ile Lys Tyr Asn Lys Ala Gly Thr Phe Glu Thr Lys Lys Ile Thr 375 Leu Pro Ser Leu Lys Ala Gln Ala Ser Ala Gly Asn Ala Asp Ala Trp 390 395 Ala Ser Ser Ser Pro Gln Ser Gly Ser Gly Ala Thr Thr Val Ser Asp 405 410 Ser Gly Asp Ser Ser Ser Gly Ser Asp Ser Asp Thr Ser Glu Thr Val 425 420 Pro Val Thr Ala Lys Gly Gly Gly Leu Tyr Thr Asp Lys Asn Leu Ser 440 Ile Thr Asn Ile Thr Gly Ile Ile Glu Ile Ala Asn Asn Lys Ala Thr 455 460 Asp Val Gly Gly Gly Ala Tyr Val Lys Gly Thr Leu Thr Cys Glu Asn 470 475 Ser His Arg Leu Gln Phe Leu Lys Asn Ser Ser Asp Lys Gln Gly Gly 485 490 Gly Ile Tyr Gly Glu Asp Asn Ile Thr Leu Ser Asn Leu Thr Gly Lys 500 505 Thr Leu Phe Gln Glu Asn Thr Ala Lys Glu Glu Gly Gly Leu Phe 520 Ile Lys Gly Thr Asp Lys Ala Leu Thr Met Thr Gly Leu Asp Ser Phe 535 540 Cys Leu Ile Asn Asn Thr Ser Glu Lys His Gly Gly Gly Ala Phe Val 550 555 Thr Lys Glu Ile Ser Gln Thr Tyr Thr Ser Asp Val Glu Thr Ile Pro 565 570 Gly Ile Thr Pro Val His Gly Glu Thr Val Ile Thr Gly Asn Lys Ser 585 Thr Gly Gly Asn Gly Gly Gly Val Cys Thr Lys Arg Leu Ala Leu Ser 600 Asn Leu Gln Ser Ile Ser Ile Ser Gly Asn Ser Ala Ala Glu Asn Gly 615 Gly Gly Ala His Thr Cys Pro Asp Ser Phe Pro Thr Ala Asp Thr Ala 630 635 Glu Gln Pro Ala Ala Ala Ser Ala Ala Thr Ser Thr Pro Lys Ser Alá 650 Pro Val Ser Thr Ala Leu Ser Thr Pro Ser Ser Ser Thr Val Ser Ser 665 Leu Thr Leu Leu Ala Ala Ser Ser Gln Ala Ser Pro Ala Thr Ser Asn 680 Lys Glu Thr Gln Asp Pro Asn Ala Asp Thr Asp Leu Leu Ile Asp Tyr 695 Val Val Asp Thr Thr Ile Ser Lys Asn Thr Ala Lys Lys Gly Gly 710 715 Ile Tyr Ala Lys Lys Ala Lys Met Ser Arg Ile Asp Gln Leu Asn Ile 730 Ser Glu Asn Ser Ala Thr Glu Ile Gly Gly Gly Ile Cys Cys Lys Glu 745 Ser Leu Glu Leu Asp Ala Leu Val Ser Leu Ser Val Thr Glu Asn Leu 760 Val Gly Lys Glu Gly Gly Leu His Ala Lys Thr Val Asn Ile Ser 775 780 Asn Leu Lys Ser Gly Phe Ser Phe Ser Asn Asn Lys Ala Asn Ser Ser 790 795

Ser Thr Gly Val Ala Thr Thr Ala Ser Ala Pro Ala Ala Ala Ala Ala 805 810 Ser Leu Gln Ala Ala Ala Ala Ala Pro Ser Ser Pro Ala Thr Pro 820 825 Thr Tyr Ser Gly Val Val Gly Gly Ala Ile Tyr Gly Glu Lys Val Thr 840 Phe Ser Gln Cys Ser Gly Thr Cys Gln Phe Ser Gly Asn Gln Ala Ile 855 860 Asp Asn Asn Pro Ser Gln Ser Ser Leu Asn Val Gln Gly Gly Ala Ile 870 875 Tyr Ala Lys Thr Ser Leu Ser Ile Gly Ser Ser Asp Ala Gly Thr Ser 885 890 Tyr Ile Phe Ser Gly Asn Ser Val Ser Thr Gly Lys Ser Gln Thr Thr 900 905 Gly Gln Ile Ala Gly Gly Ala Ile Tyr Ser Pro Thr Val Thr Leu Asn 920 Cys Pro Ala Thr Phe Ser Asn Asn Thr Ala Ser Ile Ala Thr Pro Lys 935 940 Thr Ser Ser Glu Asp Gly Ser Ser Gly Asn Ser Ile Lys Asp Thr Ile 950 955 Gly Gly Ala Ile Ala Gly Thr Ala Ile Thr Leu Ser Gly Val Ser Arg 965 970 Phe Ser Gly Asn Thr Ala Asp Leu Gly Ala Ala Ile Gly Thr Leu Ala 980 985 Asn Ala Asn Thr Pro Ser Ala Thr Ser Gly Ser Gln Asn Ser Ile Thr 1000 Glu Lys Ile Thr Leu Glu Asn Gly Ser Phe Ile Phe Glu Arg Asn Gln 1015 1020 Ala Asn Lys Arg Gly Ala Ile Tyr Ser Pro Ser Val Ser Ile Lys Gly 1030 1035 Asn Asn Ile Thr Phe Asn Gln Asn Thr Ser Thr His Asp Gly Ser Ala 1045 1050 Ile Tyr Phe Thr Lys Asp Ala Thr Ile Glu Ser Leu Gly Ser Val Leu 1060 \ 1065 1070 Phe Thr Gly Asn Asn Val Thr Ala Thr Gln Ala Ser Ser Ala Thr Ser 1080 1085 Gly Gln Asn Thr Asn Thr Ala Asn Tyr Gly Ala Ala Ile Phe Gly Asp 1095 1100 Pro Gly Thr Thr Gln Ser Ser Gln Thr Asp Ala Ile Leu Thr Leu Leu 1110 1115 Ala Ser Ser Gly Asn Ile Thr Phe Ser Asn Asn Ser Leu Gln Asn Asn 1125 1130 Gln Gly Asp Thr Pro Ala Ser Lys Phe Cys Ser Ile Ala Gly Tyr Val 1145 Lys Leu Ser Leu Gln Ala Ala Lys Gly Lys Thr Ile Ser Phe Phe Asp 1160 1165 Cys Val His Thr Ser Thr Lys Lys Thr Gly Ser Thr Gln Asn Val Tyr 1175 1180 Glu Thr Leu Asp Ile Asn Lys Glu Glu Asn Ser Asn Pro Tyr Thr Gly 1190 1195 Thr Ile Val Phe Ser Ser Glu Leu His Glu Asn Lys Ser Tyr Ile Pro 1205 1210 Gln Asn Ala Ile Leu His Asn Gly Thr Leu Val Leu Lys Glu Lys Thr 1220 1225 1230 Glu Leu His Val Val Ser Phe Glu Gln Lys Glu Gly Ser Lys Leu Ile 1235 1240 1245 Met Glu Pro Gly Ala Val Leu Ser Asn Gln Asn Ile Ala Asn Gly Ala 1255 1250 1260

Leu Ala Ile Asn Gly Leu Thr Ile Asp Leu Ser Ser Met Gly Thr Pro 1275 1270 Gln Ala Gly Glu Ile Phe Ser Pro Pro Glu Leu Arg Ile Val Ala Thr 1285 1290 Thr Ser Ser Ala Ser Gly Gly Ser Gly Val Ser Ser Ser Ile Pro Thr 1300 1305 1310 Asn Pro Lys Arg Ile Ser Ala Ala Val Pro Ser Gly Ser Ala Ala Thr 1315 1320 Thr Pro Thr Met Ser Glu Asn Lys Val Phe Leu Thr Gly Asp Leu Thr 1330 1335 1340 Leu Ile Asp Pro Asn Gly Asn Phe Tyr Gln Asn Pro Met Leu Gly Ser 1350 1355 Asp Leu Asp Val Pro Leu Ile Lys Leu Pro Thr Asn Thr Ser Asp Val 1365 1370 Gln Val Tyr Asp Leu Thr Leu Ser Gly Asp Leu Phe Pro Gln Lys Gly 1380 1385 Tyr Met Gly Thr Trp Thr Leu Asp Ser Asn Pro Gln Thr Gly Lys Leu 1395 1400 1405 Gln Ala Arg Trp Thr Phe Asp Thr Tyr Arg Arg Trp Val Tyr Ile Pro 1410 1415 1420 Arg Asp Asn His Phe Tyr Ala Asn Ser Ile Leu Gly Ser Gln Asn Ser 1430 1435 1440 Met Ile Val Val Lys Gln Gly Leu Ile Asn Asn Met Leu Asn Asn Ala 1445 1450 Arg Phe Asp Asp Ile Ala Tyr Asn Asn Phe Trp Val Ser Gly Val Gly 1460 1465 Thr Phe Leu Ala Gln Gln Gly Thr Pro Leu Ser Glu Glu Phe Ser Tyr 1480 1485 Tyr Ser Arg Gly Thr Ser Val Ala Ile Asp Ala Lys Pro Arg Gln Asp 1495 1500 Phe Ile Leu Gly Ala Ala Phe Ser Lys Ile Val Gly Lys Thr Lys Ala 1510 1515 Ile Lys Lys Met His Asn Tyr Phe His Lys Gly Ser Glu Tyr Ser Tyr 1530 1525 Gln Ala Ser Val Tyr Gly Gly Lys Phe Leu Tyr Phe Leu Leu Asn Lys 1540 1545 Gln His Gly Trp Ala Leu Pro Phe Leu Ile Gln Gly Val Val Ser Tyr 1560 Gly His Ile Lys His Asp Thr Thr Thr Leu Tyr Pro Ser Ile His Glu 1575 1580 Arg Asn Lys Gly Asp Trp Glu Asp Leu Gly Trp Leu Ala Asp Leu Arg 1590 1595 Ile Ser Met Asp Leu Lys Glu Pro Ser Lys Asp Ser Ser Lys Arg Ile 1610 1605 Thr Val Tyr Gly Glu Leu Glu Tyr Ser Ser Ile Arg Gln Lys Gln Phe 1620 1625 Thr Glu Ile Asp Tyr Asp Pro Arg His Phe Asp Asp Cys Ala Tyr Arg 1640 1645 Asn Leu Ser Leu Pro Val Gly Cys Ala Val Glu Gly Ala Ile Met Asn 1660 1655 Cys Asn Ile Leu Met Tyr Asn Lys Leu Ala Leu Ala Tyr Met Pro Ser 1670 1675 Ile Tyr Arg Asn Asn Pro Val Cys Lys Tyr Arg Val Leu Ser Ser Asn 1685 1690 Glu Ala Gly Gln Val Ile Cys Gly Val Pro Thr Arg Thr Ser Ala Arg 1705 1710 Ala Glu Tyr Ser Thr Gln Leu Tyr Leu Gly Pro Phe Trp Thr Leu Tyr 1720 1715

83

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					cccctgaaag		360
					gtgatacaat		420
•					gaaatcctta		480
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					gagccattag		600
					tggacaacat		660
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					tctataacaa		840
					gagcaattaa		900
					gtgacaatat		960
					ataatggccc		1020
					tagacggaac		1080
					atattgtgac		1140
					gaaatgcaat		1200
					aaaatttaat		1260
					ataaggaagc		- 1320
					attttcatca		1380
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<sup>&</sup>lt;210> 184

<sup>&</sup>lt;211> 2547

<sup>&</sup>lt;212> DNA

<sup>&</sup>lt;213> Chlamydia

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			aggagctatt				360
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			taagttagat				480
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			ctgttcggga				660
			aggagatatt				720
			ttttgcaggt				780
			tttccacgac				840
			aatcaatagt				900
			taaagttcct				960
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			agcagaaatc				1140
			tgctcaaaca				1200
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			caatccttat				2160
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			agatagtcag				180
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	tcttcccaag	ggttaatttg	tagttttacg	agcagcaacc	ttgattctcc	ccgtgacgga	360

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89

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 Lys Asn Gly Ala Gln Ala Gly Ser Asn Asn Ser Gly Ser Val Ser Phe
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94

Cys Leu Ala Gly Glu Ile Gly Ala Gly Leu Pro Ile Val Ile Thr Pro 855 Ser Lys Leu Tyr Leu Asn Glu Leu Arg Pro Phe Val Gln Ala Glu Phe 870 875 Ser Tyr Ala Asp His Glu Ser Phe Thr Glu Glu Gly Asp Gln Ala Arg 885 890 Ala Phe Lys Ser Gly His Leu Leu Asn Leu Ser Val Pro Val Gly Val 905 900 Lys Phe Asp Arg Cys Ser Ser Thr His Pro Asn Lys Tyr Ser Phe Met 920 925 Ala Ala Tyr Ile Cys Asp Ala Tyr Arg Thr Ile Ser Gly Thr Glu Thr 935 . 940 Thr Leu Leu Ser His Gln Glu Thr Trp Thr Thr Asp Ala Phe His Leu 945 950 955 Ala Arg His Gly Val Val Val Arg Gly Ser Met Tyr Ala Ser Leu Thr 970 975 965 Ser Asn Ile Glu Val Tyr Gly His Gly Arg Tyr Glu Tyr Arg Asp Ala 980 985 Ser Arg Gly Tyr Gly Leu Ser Ala Gly Ser Lys Val Arg Phe 1000 <210> 191 <211> 977

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	Val	Phe	Tyr 275	Asn	Asn	Arg	Cys	Phe 280	Lys	Asn	Val	Glu	Thr 285	Ala	Ser	Ser
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	-	_	_		325	Tyr				330				_	335	_
•			_	340		Asn			345			_	_	350		
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Ile Ala Thr Gln Leu Leu Ala Ser Lys Gly Ile Trp Phe Gly Ser Gly
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Ser Pro Ser Ser Arg His Ala Met Ser Tyr Lys Ile Ser Gln Gln Thr
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Phe
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<213> Chlamydia

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	Asn	Phe 130	Ser	Asp	Ile	Tyr	Gly 135	Gly	Ala	Ile	Phe	Thr 140	Gly	Ser	Leu	Arg
	Glu 145	Glu	Asp	Lys	Leu	Asp 150	Gly	Gln	Ile	Pro	Glu 155	Val	Leu	Ile	Ser	Gly 160
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				180					185					190	Leu	
			195					200					205		Ala	
•		210					215					220			Leu	
	225		-	_	_	230			-	_	235				Arg	240
		_		_	245		-			250	_				Ile 255 Ala	
				260					265					270	Leu	
			275				-	280					285		Leu	
		290	_				295	_				300			Ser	
	305 Glu	Leu	Leu	Asn	Gly	310 Ala	Thr	Leu	Cys	Ser	315 Tyr	Gly	Phe	Lys	Gln	320 Asp
	Ala	Gly	Ala	_	325 Leu	Val	Leu	Ala		330 Gly	Ser	Lys	Leu		335 Ile	Leu
	Asp	Ser	_	340 Thr	Pro	Val	Gln		345 His	Ala	Ile	Ser		350 Pro	Glu	Ala
	Glu		355 Glu	Ser	Ser	Ser	Glu 375	360 Pro	Glu	Gly	Ala	His 380	365 Ser	Leu	Trp	Ile
	Ala 385	370 Lys	Asn	Ala	Gln	Thr		Val	Pro	Met	Val 395		Ile	His	Thr	Ile 400
		Val	Asp	Leu	Ala 405		Phe	Ser	Ser	Ser 410		Gln	Glu	Gly	Thr 415	
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	Glu	Leu	Asn 435	Leu	Glu	Leu	Val	Asn 440	Thr	Thr	Gly	Thr	Gly 445	Tyr	Glu	Asn
		450			_		455		_			460			Phe	
	465					470					475				Val	480
					485					490					Thr 495	
	_			500		_			505	_				510	Thr	
			515					520		_		_	525		Lys	
		530,					535					540			Arg	
	545		-1-			550					555					560
		Phe	Asp	Tyr	Ser 565		Asn	Val	Trp	Gly 570		Ala	Phe	Gly	Gly 575	
	Arg	Thr	Leu	Ser 580	Ala	Glu	Asn	Leu	Val 585	Ala	Ile	qaA	Gly	Tyr 590	Lys	Gly

Ala Tyr Gly Gly Ala Ser Ala Gly Val Asp Ile Gln Leu Met Glu Asp 600 Phe Val Leu Gly Val Ser Gly Ala Ala Phe Leu Gly Lys Met Asp Ser 620 615 Gln Lys Phe Asp Ala Glu Val Ser Arg Lys Glý Val Val Gly Ser Val 630 635 Tyr Thr Gly Phe Leu Ala Gly Ser Trp Phe Phe Lys Gly Gln Tyr Ser 645 650 Leu Gly Glu Thr Gln Asn Asp Met Lys Thr Arg Tyr Gly Val Leu Gly 665 Glu Ser Ser Ala Ser Trp Thr Ser Arg Gly Val Leu Ala Asp Ala Leu 680 685 Val Glu Tyr Arg Ser Leu Val Gly Pro Val Arg Pro Thr Phe Tyr Ala 695 700 Leu His Phe Asn Pro Tyr Val Glu Val Ser Tyr Ala Ser Met Lys Phe 715 Pro Gly Phe Thr Glu Gln Gly Arg Glu Ala Arg Ser Phe Glu Asp Ala 725 730 Ser Leu Thr Asn Ile Thr Ile Pro Leu Gly Met Lys Phe Glu Leu Ala 745 Phe Ile Lys Gly Gln Phe Ser Glu Val Asn Ser Leu Gly Ile Ser Tyr 760 Ala Trp Glu Ala Tyr Arg Lys Val Glu Gly Gly Ala Val Gln Leu Leu 770 775 780 Glu Ala Gly Phe Asp Trp Glu Gly Ala Pro Met Asp Leu Pro Arg Gln 790 795 Glu Leu Arg Val Ala Leu Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe 805 810 Ser Thr Val Leu Gly Leu Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr 825 Asp Ser Lys Leu Gly Tyr Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe 840

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<211> 778

<212> PRT

<213> Chlamydia

<400> 193

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155 145 150 Lys Ile Lys Gly Gly Leu Glu Phe Ala Ser Cys Ser Ser Leu Glu Gln 170 175 165 Gly Gly Ala Cys Ala Ala Gln Ser Ile Leu Ile His Asp Cys Gln Gly 185 190 180 Leu Gln Val Lys His Cys Thr Thr Ala Val Asn Ala Glu Gly Ser Ser 200 Ala Asn Asp His Leu Gly Phe Gly Gly Gly Ala Phe Phe Val Thr Gly 215 220 Ser Leu Ser Gly Glu Lys Ser Leu Tyr Met Pro Ala Gly Asp Met Val 225 230 235 Val Ala Asn Cys Asp Gly Ala Ile Ser Phe Glu Gly Asn Ser Ala Asn 245 250 255 Phe Ala Asn Gly Gly Ala Ile Ala Ala Ser Gly Lys Val Leu Phe Val 260 265 270 Ala Asn Asp Lys Lys Thr Ser Phe Ile Glu Asn Arg Ala Leu Ser Gly 280 275 Gly Ala Ile Ala Ala Ser Ser Asp Ile Ala Phe Gln Asn Cys Ala Glu 295 300 Leu Val Phe Lys Gly Asn Cys Ala Ile Gly Thr Glu Asp Lys Gly Ser 310 · 315 Leu Gly Gly Gly Ala Ile Ser Ser Leu Gly Thr Val Leu Leu Gln Gly 325 330 335 Asn His Gly Ile Thr Cys Asp Lys Asn Glu Ser Ala Ser Gln Gly Gly 340 345 350 Ala Ile Phe Gly Lys Asn Cys Gln Ile Ser Asp Asn Glu Gly Pro Val 360 365 Val Phe Arg Asp Ser Thr Ala Cys Leu Gly Gly Gly Ala Ile Ala Ala 375 380 Gln Glu Ile Val Ser Ile Gln Asn Asn Gln Ala Gly Ile Ser Phe Glu 390 395 Gly Gly Lys Ala Ser Phe Gly Gly Gly Ile Ala Cys Gly Ser Phe Ser 405 410 415 Ser Ala Gly Gly Ala Ser Val Leu Gly Thr Ile Asp Ile Ser Lys Asn 420 425 430 Leu Gly Ala Ile Ser Phe Ser Arg Thr Leu Cys Thr Thr Ser Asp Leu 435 440 Gly Gln Met Glu Tyr Gln Gly Gly Ala Leu Phe Gly Glu Asn Ile 455 Ser Leu Ser Glu Asn Ala Gly Val Leu Thr Phe Lys Asp Asn Ile Val 470 475 Lys Thr Phe Ala Ser Asn Gly Lys Ile Leu Gly Gly Gly Ala Ile Leu 490 485 Ala Thr Gly Lys Val Glu Ile Thr Asn Asn Ser Gly Gly Ile Ser Phe 500 505 Thr Gly Asn Ala Arg Ala Pro Gln Ala Leu Pro Thr Gln Glu Glu Phe 520 525 Pro Leu Phe Ser Lys Lys Glu Gly Arg Pro Leu Ser Ser Gly Tyr Ser 535 540 Gly Gly Ala Ile Leu Gly Arg Glu Val Ala Ile Leu His Asn Ala 550 555 560 Ala Val Val Phe Glu Gln Asn Arg Leu Gln Cys Ser Glu Glu Glu Ala 570 Thr Leu Leu Gly Cys Cys Gly Gly Gly Ala Val His Gly Met Asp Ser 585 Thr Ser Ile Val Gly Asn Ser Ser Val Arg Phe Gly Asn Asn Tyr Ala 600 Met Gly Gln Gly Val Ser Gly Gly Ala Leu Leu S r Lys Thr Val Gln

PCT/US00/32919

100

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Gly Gly Gly Ala Leu Gln Ala Ser Glu Gly Asn Cys Glu Leu Val Asp
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Asn Gly Tyr Val Leu Phe Arg Asp Asn Arg Gly Arg Val Tyr Gly Gly
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Ala Ile Ser Cys Leu Arg Gly Asp Val Val Ile Ser Gly Asn Lys Gly
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Arg Val Glu Phe Lys Asp Asn Ile Ala Thr Arg Leu Tyr Val Glu Glu
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Thr Val Glu Lys Val Glu Glu Val Glu Pro Ala Pro Glu Gln Lys Asp
       710 715
Asn Asn Glu Leu Ser Phe Leu Gly Ser Val Glu Gln Ser Phe Ile Thr
         725 730 735
Ala Ala Asn Gln Ala Leu Phe Ala Ser Glu Asp Gly Asp Leu Ser Pro
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           40
Lys Thr Leu Phe Asn Leu Asp Ser Gly Ser Ser Arg Arg Thr Val Thr
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                                   60
Phe Ser Gly Asn Thr Val Ser Ser Gln Ser Thr Thr Gly Gln Val Ala
                              75
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Gly Gly Ala Ile Tyr Ser Pro Thr Val Thr Ile Ala Thr Pro Val Val
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Phe Ser Lys Asn Ser Ala Thr Asn Asn Ala Asn Asn Ala Thr Asp Thr
                          105
Gln Arg Lys Asp Thr Phe Gly Gly Ala Ile Gly Ala Thr Ser Ala Val
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                                       125
Ser Leu Ser Gly Gly Ala His Phe Leu Glu Asn Val Ala Asp Leu Gly
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Ser Ala Ile Gly Leu Val Pro Asp Thr Gln Asn Thr Glu Thr Val Lys
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               150
Leu Glu Ser Gly Ser Tyr Tyr Phe Glu Lys Asn Lys Ala Leu Lys Arg
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Ala Thr Ile Tyr Ala Pro Val Val Ser Ile Lys Ala Tyr Thr Ala Thr
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                          185
Phe Asn Gln Asn Arg Ser Leu Glu Glu Gly Ser Ala Ile Tyr Phe Thr
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                                       205
Lys Glu Ala Ser Ile Glu Ser Leu Gly Ser Val Leu Phe Thr Gly Asn
  210 215
                                   220
Leu Val Thr Pro Thr Leu Ser Thr Thr Thr Glu Gly Thr Pro Ala Thr
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230

Thr	Ser	Gly	Asp	Val 245	Thr	Lys	Tyr	Gly	Ala 250	Ala	Ile	Phe	Gly	Gln 255	Ile
Ala	Ser	Ser	Asn 260	Gly	Ser	Gln	Thr	Asp 265	Asn	Leu	Pro	Leu	Lys 270	Leu	Ile
Ala	Ser	Gly 275	Gly	Asn	Ile	Сув	Phe 280	Arg	Asn	Asn	Glu	Tyr 285	Arg	Pro	Thr
Ser	Ser 290	Asp	Thr	Gly	Thr	Ser 295	Thr	Phe	Cys	Ser	Ile 300	Ala	Gly	Asp	Val
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			340		Asn			345					350		
		355	_		Ile		360					365			
	370				Asn	375					380				
385					Leu 390					395					400
Ser	Ser	Leu	Val	Met 405	Thr	Pro	Gly	Ser	Val 410	Leu	Ser	Asn	GIn	Thr 415	Val
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		435		_	Ile		440					445			
	450			_	Thr	455					460				
465	_				Asn 470					475					480
		_		485	Asn				490	•				495	
			500		Ala			505					510		
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Ser	Asn 530	Gln	Val	Ile	Leu	Gly 535	Gly	Glu	Ile	Lys	Leu 540	Ile	Asp	Pro	Asn
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Phe 625	Tyr	Ala	Asn	Ser	Ile 630	Leu	Gly	Ser	Gln	Met 635	Ser	Met	Val	Thr	Val 640
Lys	Gln	Gly	Leu	Leu 645	Asn	Asp	Lys	Met	Asn 650	Leu	Ala	Arg	Phe	Asp 655	Glu
Val	Ser	Tyr	Asn 660	Asn	Leu	Trp	Ile	Ser 665	Gly	Leu	Gly	Thr	Met 670	Leu	Ser
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Ala	Ser 690	Val	Ala	Leu	Asp	Ala 695	Lys	Pro	Ala	His	Asp 700	Val	Ile	Val	Gly

Ala Ala Phe Ser Lys Met Ile Gly Lys Thr Lys Ser Leu Lys Arg Glu

715 710 Asn Asn Tyr Thr His Lys Gly Ser Glu Tyr Ser Tyr Gln Ala Ser Val 725 730 Tyr Gly Gly Lys Pro Phe His Phe Val Ile Asn Lys Lys Thr Glu Lys 745 Ser Leu Pro Leu Leu Gln Gly Val Ile Ser Tyr Gly Tyr Ile Lys 760 His Asp Thr Val Thr His Tyr Pro Thr Ile Arg Glu Arg Asn Gln Gly 775 780 Glu Trp Glu Asp Leu Gly Trp Leu Thr Ala Leu Arg Val Ser Ser Val 790 795 Leu Arg Thr Pro Ala Gln Gly Asp Thr Lys Arg Ile Thr Val Tyr Gly 810 Glu Leu Glu Tyr Ser Ser Ile Arg Gln Lys Gln Phe Thr Glu Thr Glu 825 Tyr Asp Pro Arg Tyr Phe Asp Asn Cys Thr Tyr Arg Asn Leu Ala Ile 840 Pro Met Gly Leu Ala Phe Glu Gly Glu Leu Ser Gly Asn Asp Ile Leu 855 Met Tyr Asn Arg Phe Ser Val Ala Tyr Met Pro Ser Ile Tyr Arg Asn Ser Pro Thr Cys Lys Tyr Gln Val Leu Ser Ser Gly Glu Gly Gly Glu 890 885 Ile Ile Cys Gly Val Pro Thr Arg Asn Ser Ala Arg Gly Glu Tyr Ser 905 Thr Gln Leu Tyr Pro Gly Pro Leu Trp Thr Leu Tyr Gly Ser Tyr Thr 920 Ile Glu Ala Asp Ala His Thr Leu Ala His Met Met Asn Cys Gly Ala Arg Met Thr Phe 945 <210> 195 <211> 821 <212> PRT <213> Chlamydia <400> 195 Met His His His His His Glu Ala Ser Ser Ile Gln Asp Gln Ile 10 Lys Asn Thr Asp Cys Asn Val Ser Lys Val Gly Tyr Ser Thr Ser Gln Ala Phe Thr Asp Met Met Leu Ala Asp Asn Thr Glu Tyr Arg Ala Ala Asp Ser Val Ser Phe Tyr Asp Phe Ser Thr Ser Ser Gly Leu Pro Arg Lys His Leu Ser Ser Ser Glu Ala Ser Pro Thr Thr Glu Gly Val Ser Ser Ser Ser Gly Glu Asn Thr Glu Asn Ser Gln Asp Ser Ala 90 Pro Ser Ser Gly Glu Thr Asp Lys Lys Thr Glu Glu Glu Leu Asp Asn 105 Gly Gly Ile Ile Tyr Ala Arg Glu Lys Leu Thr Ile Ser Glu Ser Gln 120 125 Asp Ser Leu Ser Asn Pro Ser Ile Glu Leu His Asp Asn Ser Phe Phe 135 140 Phe Gly Glu Gly Glu Val Ile Phe Asp His Arg Val Ala Leu Lys Asn

	145					150					155					160
	Glv	Gly	Ala	Ile	Tyr	Gly	Glu	Lys	Glu	Val	Val	Phe	Glu	Asn	Ile	Lys
	,				165		-	-4 -		170					175	•
	Car	T.611	T.e.11	Val		Val	Asn	Tle	Ser		Glu	Tivs	Glv	Glv		Val
	001			180					185			-1-	1	190		
	(TIn	77.	T		3	Val	C0~	T 0		7 ~~	37-3	Thr	Clu		The	Dho
	Tyr	Ald	-	GIU	Arg	val	Ser		GIU	ASII	vai	1111	205	ALG	1111	FILE
	_	_	195					200	<b>~</b> 3	<b>a</b> 3	<b>-</b> 1	-1.			<b>01</b>	<b>03</b>
	Ser		Asn	GIA	GTÅ	Glu		GIY	GTA	GTA	GIA		Tyr	ser	GIU	GIn
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	225					230					235					240
•	Ala	Gly	Ala	Thr	Ala	Val	Lys	Gln	Cys	Leu	Asp	Glu	Glu	Met	<b>Ile</b>	Val
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	Thr	Pro	Glu	Thr	Glu	Gln	Thr	Lvs	Ser	Asn	Gly	Asn	Gln	Asp	Gly	Ser
			275					280			•		285	•	-	
	Ser	Glu		Lve	Δen	Thr	Gln		Ser	Glu	Ser	Pro	-	Ser	Thr	Pro
	Ser	290	1111	цуз	лар	1111	295	val	561	JIU	UCL	300	014			
	C ~ ~		3	B ===	1/01	Leu		T 110	C1.,	C1	C111		Tarr	The	Gl.	Lve
		Pro	Asp	Asp	var		GIA	гля	GIA	GIY		116	IYI	1111	GIU	
	305	_				310		_,			315	<b>-</b>	D1		<b>a</b>	320
	ser	Leu	Thr	ire		Gly	TIE	Thr	GIY		тте	Asp	Pne	vai		ASI
	_				325					330		_		_	335	_
	Ile	Ala	Thr		Ser	Gly	Ala	Gly		Phe	Thr	Lys	Glu		Leu	Ser
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	Cys	Thr	Asn	Thr	Asn	Ser	Leu	Gln	Phe	Leu	Lys	Asn	Ser	Ala	Gly	Gln
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	His	Gly	Gly	Gly	Ala	Tyr	Val	Thr	Gln	Thr	Met	Ser	Val	Thr	Asn	Thr
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	Thr	Ser	Glu	Ser	Ile	Thr	Thr	Pro	Pro	Leu	Val	Gly	Glu	Val	Ile	Phe
	385					390					395	-				400
		Glu	Asn	Thr	Ala	Lys	Glv	His	Glv	Glv	Glv	Ile	Cvs	Thr	Asn	Lvs
					405	-1-			2	410			•		415	•
	T.e.11	Ser	T.e.13	Ser		Leu	Lvg	Thr	Val		Leu	Thr	Lvs	Asn		Ala
			200	420			-,-		425				-,-	430		•
	Larg	G111	Car		Gly	Ala	716	Dhe		\an	T.e.11	a l a	Ser		Pro	Thr
	пуз	GIU	435	GIY	GLY	AIG	116	440	1111	тэр	nc a	AIG	445	110	110	1111
	ml	*		D	<b>~1</b>		0		D	C	C	C		Dwa	A 1 -	00-
	Inr	_	THE	PIO	GIU	Ser		Inr	PIO	ser	ser		Sei	PIO	AIG	ser
	1	450		•			455		_		•	460	<b>51</b>	<b>51.</b> -		<b>a</b>
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	Thr	Ala	Glu	Pro		Ala	Pro	Ser	Leu		Glu	Ala	Glu	Ser		Gin
					485					490					495	
	Thr	Asp	Gln	Thr	Glu	Thr	Ser	Asp	Thr	Asn	Ser	Asp	Ile	Asp	Val	Ser
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	Lvs	Glv	Glv	Ala	Ile	Tyr	Glv	Lvs	Lvs	Ala	Lvs	Leu	Ser	Arg	Ile	Asn
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	545		014	204		550		<b>DC1</b>		<b>V</b> 2.11	555	• • • •	0_,	0-7	0-1	560
		T 011	mb.~	C7.,	Car		@1 <sub>11</sub>	Dha	λen	71-		C111	802	Tan	Len	
	Cys	ьеи	Int	GIU		Val	GIU	FIIE	ASP		ire	GIA	Ser	Leu		361
		_	_	_	565			~7	٠.	570		,		_	575	<b></b>
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	_	_		580		_	_	_	585					590		
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	Val	Lys	Ala	Ile	Val	Glu	Ser	Thr	Pro	Glu	Ala	Pro	Glu	Glu	Ile	Pro

104

615 620 Pro Val Glu Gly Glu Ger Thr Ala Thr Glu Asn Pro Asn Ser Asn 630 635 Thr Glu Gly Ser Ser Ala Asn Thr Asn Leu Glu Gly Ser Gln Gly Asp 645 650 Thr Ala Asp Thr Gly Thr Gly Val Val Asn Asn Glu Ser Gln Asp Thr 660 665 Ser Asp Thr Gly Asn Ala Glu Ser Gly Glu Gln Leu Gln Asp Ser Thr 680 Gln Ser Asn Glu Glu Asn Thr Leu Pro Asn Ser Ser Ile Asp Gln Ser 700 695 Asn Glu Asn Thr Asp Glu Ser Ser Asp Ser His Thr Glu Glu Ile Thr 710 715 Asp Glu Ser Val Ser Ser Ser Ser Lys Ser Gly Ser Ser Thr Pro Gln 725 730 · Asp Gly Gly Ala Ala Ser Ser Gly Ala Pro Ser Gly Asp Gln Ser Ile 740 745 Ser Ala Asn Ala Cys Leu Ala Lys Ser Tyr Ala Ala Ser Thr Asp Ser 760 Ser Pro Val Ser Asn Ser Ser Gly Ser Asp Val Thr Ala Ser Ser Asp 775 780 Asn Pro Asp Ser Ser Ser Gly Asp Ser Ala Gly Asp Ser Glu Gly 790 795 Pro Thr Glu Pro Glu Ala Gly Ser Thr Thr Glu Thr Pro Thr Leu Ile 810 Gly Gly Gly Ala Ile 820 <210> 196 <211> 525 <212> PRT <213> Chlamydia Met His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala 25 Ile Ala Gly Gln Ile Lys Leu Pro Thr Val His Ile Gly Pro Thr Ala 40 Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val 55 Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr 70 75 Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr 85 90 Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser 105 Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr 120 Leu Ala Glu Gly Pro Pro Ala Glu Phe Pro Leu Val Pro Arg Gly Ser 135 Pro Leu Pro Val Gly Asn Pro Ala Glu Pro Ser Leu Leu Ile Asp Gly 155 150 Thr Met Trp Glu Gly Ala Ser Gly Asp Pro Cys Asp Pro Cys Ala Thr 165 170 Trp Cys Asp Ala Ile Ser Ile Arg Ala Gly Tyr Tyr Gly Asp Tyr Val

105

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Ala Ala Thr Pro Thr Gln Ala Ile Gly Asn Ala Ser Asn Thr Asn Gln
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Pro Glu Ala Asn Gly Arg Pro Asn Ile Ala Tyr Gly Arg His Met Gln
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Gly Ile Thr Gln Gly Val Val Glu Phe Tyr Thr Asp Thr Ser Phe Ser
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Trp Ser Val Gly Ala Arg Gly Ala Leu Trp Glu Cys Gly Cys Ala Thr
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Leu Gly Ala Glu Phe Gln Tyr Ala Gln Ser Asn Pro Lys Ile Glu Met
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Leu Asn Val Thr Ser Ser Pro Ala Gln Phe Val Ile His Lys Pro Arg
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Thr Thr Glu Ala Thr Asp Thr Lys Ser Ala Thr Ile Lys Tyr His Glu
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Trp Gln Val Gly Leu Ala Leu Ser Tyr Arg Leu Asn Met Leu Val Pro
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Tyr Ile Gly Val Asn Trp Ser Arg Ala Thr Phe Asp Ala Asp Thr Ile
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Arg Ile Ala Gln Pro Lys Leu Lys Ser Glu Ile Leu Asn Ile Thr Thr
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Trp Asn Pro Ser Leu Ile Gly Ser Thr Thr Ala Leu Pro Asn Asn Ser
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                                   460
Gly Lys Asp Val Leu Ser Asp Val Leu Gln Ile Ala Ser Ile Gln Ile
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Asn Lys Met Lys Ser Arg Lys Ala Cys Gly Val Ala Val Gly Ala Thr
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 His Lys Arg Arg Ala Ala Ala Ala Val Cys Ser Ile Ile Gly Gly Ile
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 Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
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 Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val
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Glu Glu Asn Ala Cys Glu Lys Lys Val Ala Gly Glu Lys Ala Lys Thr
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Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met
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Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
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Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arg 75 Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr 90 95 85 Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln 100 105 Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser 120 125 115 His Lys Arg Arg Ala Ala Ala Val Cys Ser Ile Ile Gly Gly Ilè 135 140 Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn 155 150 Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met 170 165 Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val 180 ` 185 190 Val Gly Ala Gly Leu Ala Ile Ser Ala Xaa Arg Ala Asp Cys Glu Ala 200 205 Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Val Pro Gly 215 220 Glu Glu Asn Ala Cys Glu Lys Lys Val Ala Gly Glu Lys Ala Lys Thr 230 235 Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu 245 250 Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met 265 260 Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile 275 280 Ile Gly Leu Cys Thr Phe Cys Ala Arg Ala 295 <210> 267 <211> 680 <212> DNA <213> Chlamydia <400> 267 tetatateca tattgatagg aaaaaaegte geagaaagat tttagetatg aegtttatee 60 gagetttagg atatteaaca gatgeagata ttattgaaga gttettttet gtagaggage 120 gttccttacg ttcagagaag gattttgtcg cgttagttgg taaagtttta gctgataacg 180 tagttgatgc ggattettea ttagtttacg ggaaagetgg agagaageta agtactgeta 240 tgctaaaacg catcttagat acgggagtcc aatctttgaa gattgctgtt ggcgcagatg 300 aaaatcaccc aattattaag atgctcgcaa aagatcctac ggattcttac gaagctgctc 360 ttaaagattt ttatcgcaga ttacgaccag gagagcctgc aactttagct aatgctcgat 420 ccacaattat gcgtttattc ttcgatgcta aacgttataa tttaggccgc gttggacgtt 480 ataaattaaa taaaaaatta ggcttcccat tagacgacga aacattatct caagtgactt 540 tgagaaaaga agatgttatc ggcgcgttga aatatttgat tcgtttgcga atgggcgatg 600 agaagacatc tatcgatgat attgaccatt tggcaaaccg acgagttcgc tctgttggag 660 aactaattca gaatcactgt 680 <210> 268 <211> 359 <212> DNA <213> Chlamydia <400> 268

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60

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WO 01/40474 PCT/US00/32919 123

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Gly Gly Ile Ala Leu Glu Gln Lys Leu Phe Lys Arg Ala Lys Glu Ser

129

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<400> 295

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Val Gly Ala Leu Val Val Val Ala Gly Val Leu Ala Leu Val Leu Cys

Ala Ser Asn Val Ile Phe Thr Val Ile Gly Ile Pro Ala Leu Ile Ile

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<211> 124

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<213> Chlamydia

131

<400> 296

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Glu Asn Pro Ser Thr Ser Phe Cys Gln Gln Val Leu Ala Asp Phe Ile 100 105 110

Gly Gly Leu Asn Asp Phe His Ala Gly Val Thr Phe Phe Ala Ile Glu 115 120 125

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<210> 298

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<212> PRT

<213> Chlamydia

<400> 298

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Val Ser Ser Val Leu Asp Asn Val Pro Leu Val Ala Ala Thr Ile Gly

Met Tyr Asp Leu Pro Met Asn Asp Pro Leu Trp Lys Leu Ile Ala Tyr

Thr Ala Gly Thr Gly Gly Ser Ile Leu Ile Ile Gly Ser Ala Ala Gly

Val Ala Tyr Met Gly Met Glu Lys Val Ser Phe Gly Trp Tyr Val Lys

His Ala Ser Trp Ile Ala Leu Ala Ser Tyr Phe Gly Gly Leu Ala Val

Tyr Phe Leu Met Glu Asn Cys Val Asn Leu Phe Val

<210> 299

<211> 361

<212> PRT

<213> Chlamydia

<400> 299

His Gln Glu Ile Ala Asp Ser Pro Leu Val Lys Lys Ala Glu Gln Gln

Ile Asn Gln Ala Gln Gln Asp Ile Gln Thr Ile Thr Pro Ser Gly Leu

Asp Ile Pro Ile Val Gly Pro Ser Gly Ser Ala Ala Ser Ala Gly Ser

Ala Ala Gly Ala Leu Lys Ser Ser Asn Asn Ser Gly Arg Ile Ser Leu Leu Leu Asp Asp Val Asp Asn Glu Met Ala Ala Ile Ala Met Gln Gly Phe Arg Ser Met Ile Glu Gln Phe Asn Val Asn Asn Pro Ala Thr Ala Lys Glu Leu Gln Ala Met Glu Ala Gln Leu Thr Ala Met Ser Asp Gln Leu Val Gly Ala Asp Gly Glu Leu Pro Ala Glu Ile Gln Ala Ile Lys Asp Ala Leu Ala Gln Ala Leu Lys Gln Pro Ser Ala Asp Gly Leu Ala Thr Ala Met Gly Gln Val Ala Phe Ala Ala Ala Lys Val Gly Gly Gly Ser Ala Gly Thr Ala Gly Thr Val Gln Met Asn Val Lys Gln Leu Tyr Lys Thr Ala Phe Ser Ser Thr Ser Ser Ser Tyr Ala Ala Ala Leu Ser Asp Gly Tyr Ser Ala Tyr Lys Thr Leu Asn Ser Leu Tyr Ser Glu Ser Arg Ser Gly Val Gln Ser Ala Ile Ser Gln Thr Ala Asn Pro Ala Leu Ser Arg Ser Val Ser Arg Ser Gly Ile Glu Ser Gln Gly Arg Ser Ala Asp Ala Ser Gln Arg Ala Ala Glu Thr Ile Val Arg Asp Ser Gln Thr Leu Gly Asp Val Tyr Ser Arg Leu Gln Val Leu Asp Ser Leu Met Ser Thr Ile Val Ser Asn Pro Gln Ala Asn Gln Glu Glu Ile Met Gln Lys Leu Thr Ala Ser Ile Ser Lys Ala Pro Gln Phe Gly Tyr Pro Ala Val Gln Asn Ser Val Asp Ser Leu Gln Lys Phe Ala Ala Gln Leu Glu Arg Glu Phe Val Asp Gly Glu Arg Ser Leu Ala Glu Ser Gln Glu Asn 325 Ala Phe Arg Lys Gln Pro Ala Phe Ile Gln Gln Val Leu Val Asn Ile

135

Ala Ser Leu Phe Ser Gly Tyr Leu Ser

<210> 300

<211> 207

<212> PRT

<213> Chlamydia

<400> 300

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Met Cys Lys Ala Glu Leu Ile Lys Lys Glu Ala Asp Ala Tyr Leu Phe 20 25 30

Cys Glu Lys Ser Gly Ile Tyr Leu Thr Lys Lys Glu Gly Ile Leu Ile
35 40 45

Pro Ser Ala Gly Ile Asp Glu Ser Asn Thr Asp Gln Pro Phe Val Leu
50 55 60

Tyr Pro Lys Asp Ile Leu Gly Ser Cys Asn Arg Ile Gly Glu Trp Leu 65 70 75 80

Arg Asn Tyr Phe Arg Val Lys Glu Leu Gly Val Ile Ile Thr Asp Ser 85 90 95

His Thr Thr Pro Met Arg Arg Gly Val Leu Gly Ile Gly Leu Cys Trp
100 105 110

Tyr Gly Phe Ser Pro Leu His Asn Tyr Ile Gly Ser Leu Asp Cys Phe 115 120 125

Gly Arg Pro Leu Gln Met Thr Gln Ser Asn Leu Val Asp Ala Leu Ala 130 135 140

Val Ala Ala Val Val Cys Met Gly Glu Gly Asn Glu Gln Thr Pro Leu 145 150 155 160

Ala Val Ile Glu Gln Ala Pro Asn Met Val Tyr His Ser Tyr Pro Thr

Ser Arg Glu Glu Tyr Cys Ser Leu Arg Ile Asp Glu Thr Glu Asp Leu 180 185 190

Tyr Gly Pro Phe Leu Gln Ala Val Thr Trp Ser Gln Glu Lys Lys 195 200 205

<210> 301

<211> 183

<212> PRT

<213> Chlamydia

<400> 301

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Ile Asp Ala Asn Gly Ile Leu His Val Ser Ala Lys Asp Ala Ala Ser

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Asp Glu Ile Gln Gln Met Ile Arg Asp Ala Glu Leu His Lys Glu Glu

Asp Lys Gln Arg Lys Glu Ala Ser Asp Val Lys Asn Glu Ala Asp Gly

Met Ile Phe Arg Ala Glu Lys Ala Val Lys Asp Tyr His Asp Lys Ile

Pro Ala Glu Leu Val Lys Glu Ile Glu Glu His Ile Glu Lys Val Arg

Gln Ala Ile Lys Glu Asp Ala Ser Thr Thr Ala Ile Lys Ala Ala Ser

Asp Glu Leu Ser Thr Arg Met Gln Lys Ile Gly Glu Ala Met Gln Ala

Gln Ser Ala Ser Ala Ala Ala Ser Ser Ala Ala Asn Ala Gln Gly Gly

Pro Asn Ile Asn Ser Glu Asp Leu Lys Lys His Ser Phe Ser Thr Arg 170

Pro Pro Ala Gly Gly Ser Ala 180

<210> 302

<211> 232

<212> PRT

<213> Chlamydia

<400> 302

Met Thr Lys His Gly Lys Arg Ile Arg Gly Ile Gln Glu Thr Tyr Asp

Leu Ala Lys Ser Tyr Ser Leu Gly Glu Ala Ile Asp Ile Leu Lys Gln

Cys Pro Thr Val Arg Phe Asp Gln Thr Val Asp Val Ser Val Lys Leu

Gly Ile Asp Pro Arg Lys Ser Asp Gln Gln Ile Arg Gly Ser Val Ser

Leu Pro His Gly Thr Gly Lys Val Leu Arg Ile Leu Val Phe Ala Ala

Gly Asp Lys Ala Ala Glu Ala Ile Glu Ala Gly Ala Asp Phe Val Gly

Ser Asp Asp Leu Val Glu Lys Ile Lys Gly Gly Trp Val Asp Phe Asp 100 105 110

Val Ala Val Ala Thr Pro Asp Met Met Arg Glu Val Gly Lys Leu Gly
115 120 125

Lys Val Leu Gly Pro Arg Asn Leu Met Pro Thr Pro Lys Ala Gly Thr 130 135 140

Val Thr Thr Asp Val Val Lys Thr Ile Ala Glu Leu Arg Lys Gly Lys 145 150 160

Ile Glu Phe Lys Ala Asp Arg Ala Gly Val Cys Asn Val Gly Val Ala 165 170 175

Lys Leu Ser Phe Asp Ser Ala Gln Ile Lys Glu Asn Val Glu Ala Leu 180 185 190

Cys Ala Ala Leu Val Lys Ala Lys Pro Ala Thr Ala Lys Gly Gln Tyr 195 200 205

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<210> 303

<211> 238

<212> PRT

<213> chlamydia

<400> 303

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Glu Pro Leu Lys Asp Gln Gln Ile Ile Leu Gly Thr Thr Ser Thr Pro
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Val Ala Ala Lys Met Thr Ala Ser Asp Gly Ile Ser Leu Thr Val Ser 65 70 75 80

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85 90 95

Lys Ala Tyr Gln Leu Ile Leu Glu Lys Leu Gly Asp Gln Ile Leu Gly
100 105 110

Gly Ile Ala Asp Thr Ile Val Asp Ser Thr Val Gln Asp Ile Leu Asp 115 120 125

Lys Ile Thr Thr Asp Pro Ser Leu Gly Leu Leu Lys Ala Phe Asn Asn 130 135 140

Phe Pro Ile Thr Asn Lys Ile Gln Cys Asn Gly Leu Phe Thr Pro Arg 145 150 155 160

Asn Ile Glu Thr Leu Leu Gly Gly Thr Glu Ile Gly Lys Phe Thr Val 165 170 175

Thr Pro Lys Ser Ser Gly Ser Met Phe Leu Val Ser Ala Asp Ile Ile 180 185 190

Ala Ser Arg Met Glu Gly Gly Val Val Leu Ala Leu Val Arg Glu Gly
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Asp Ser Lys Pro Tyr Ala Ile Ser Tyr Gly Tyr Ser Ser Gly Val Pro 210 215 220

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<210> 304

<211> 133

<212> PRT

<213> Chlamydia

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Asn Lys Met Ala Arg Val Val Asn Lys Thr Lys Gly Met Asp Lys Thr 35 40 45

Val Lys Val Ala Lys Ser Ala Ala Glu Leu Thr Ala Asn Ile Leu Glu 50 55 60

Gln Ala Gly Gly Ala Gly Ser Ser Ala His Ile Thr Ala Ser Gln Val 65 70 75 80

Ser Lys Gly Leu Gly Asp Thr Arg Thr Val Val Ala Leu Gly Asn Ala 85 90 95

Phe Asn Gly Ala Leu Pro Gly Thr Val Gln Ser Ala Gln Ser Phe Phe 100 105 110

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Leu Thr Ala Asp Leu 130

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<211> 125

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<211> 619

<212> PRT

<213> Chlamydia trachomatis

<400> 309

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Leu Ala Ile Ser Gly His Lys Gln Gly Lys Asp Arg Asp Thr Phe Thr 215 220 Met Ile Ser Ser Cys Pro Glu Gly Thr Asn Tyr Ile Ile Asn Arg Lys 235 230 Leu Ile Leu Ser Asp Phe Ser Leu Leu Asn Lys Val Ser Ser Gly Gly 245 250 Ala Phe Arg Asn Leu Ala Gly Lys Ile Ser Phe Leu Gly Lys Asn Ser 265 Ser Ala Ser Ile His Phe Lys His Ile Asn Ile Asn Gly Phe Gly Ala 280 275 Gly Val Phe Ser Glu Ser Ser Ile Glu Phe Thr Asp Leu Arg Lys Leu 295 300 Val Ala Phe Gly Ser Glu Ser Thr Gly Gly Ile Phe Thr Ala Lys Glu 315 310 Asp Ile Ser Phe Lys Asn Asn His His Ile Ala Phe Arg Asn Asn Ile 325 330 Thr Lys Gly Asn Gly Gly Val Ile Gln Leu Gln Gly Asp Met Lys Gly 345 Ser Val Ser Phe Val Asp Gln Arg Gly Ala Ile Ile Phe Thr Asn Asn 360 Gln Ala Val Thr Ser Ser Met Lys His Ser Gly Arg Gly Gly Ala 375 380 Ile Ser Gly Asp Phe Ala Gly Ser Arg Ile Leu Phe Leu Asn Asn Gln 390 395 Gln Ile Thr Phe Glu Gly Asn Ser Ala Val His Gly Gly Ala Ile Tyr 405 410 Asn Lys Asn Gly Leu Val Glu Phe Leu Gly Asn Ala Gly Pro Leu Ala 425 420 Phe Lys Glu Asn Thr Thr Ile Ala Asn Gly Gly Ala Ile Tyr Thr Ser 440 Asn Phe Lys Ala Asn Gln Gln Thr Ser Pro Ile Leu Phe Ser Gln Asn 455 His Ala Asn Lys Lys Gly Gly Ala Ile Tyr Ala Gln Tyr Val Asn Leu 470 Glu Gln Asn Gln Asp Thr Ile Arg Phe Glu Lys Asn Thr Ala Lys Glu 490 Gly Gly Gly Ala Ile Thr Ser Ser Gln Cys Ser Ile Thr Ala His Asn 505 Thr Ile Thr Phe Ser Asp Asn Ala Ala Gly Asp Leu Gly Gly Ala . 520 525 Ile Leu Leu Glu Gly Lys Lys Pro Ser Leu Thr Leu Ile Ala His Ser 535 Gly Asn Ile Ala Phe Ser Gly Asn Thr Met Leu His Ile Thr Lys Lys 550 555 Ala Ser Leu Asp Arg His Asn Ser Ile Leu Ile Lys Glu Ala Pro Tyr 570 Lys Ile Gln Leu Ala Ala Asn Lys Asn His Ser Ile His Phe Phe Asp 585 Pro Val Met Ala Leu Ser Ala Ser Ser Pro Ile Gln Ile Asn Ala 600 Pro Glu Tyr Glu Thr Pro Phe Phe Ser Pro Lys 615

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<sup>&</sup>lt;211> 39

<sup>&</sup>lt;212> DNA

<sup>&</sup>lt;213> Chlamydia trachomatis

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<212> PRT

<213> Chlamydia trachomatis

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	Asn	Leu 210		Ser	Asn	Gly	Thr 215	Gly	Ala	Сув	Thr	Ile 220	Ser	Gly	Asn	Thr
	Gln 225	Thr	Gln	Ile	Phe	Ser 230	Asn	Ser	Val	Asn	Thr 235	Thr	Ala	Asp	Ser	Gly 240
		Ala	Phe	Asp	Met 245	Val	Thr	Thr	Ser	Phe 250	Thr	Ala	Ser	Asp	Asn 255	Ala
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		Leu			325					330					335	
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		Ile		420					425					430		
		Gly	435					440					445			
		Thr 450					455					460				
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		Asp		_	485		_			490					495	
		Trp	_	500					505	_		_		510		
	-	Ile	515					520					525			
		Thr 530		_	•		535		_	_	_	540				
	545	Asn				550		_			555					560
	_	Thr	_		565					570		_			575	
				580					585					590		
		Val Gln	595					600		_			605			
		610 Ile				_	615	_				620	-			
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1980

147

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_	_	Val		85			_	_	90					95	
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		Ala	-	165					170					175	
		Glu	180					185					190		
		Leu 195					200					205	_	_	
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PCT/US00/32919 WO 01/40474

149

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 gcagcetece attatgaaaa tgggtcaata tttggagtgg cttttggaca actetatggt
                                                                       1500
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gatcattcga	aatgtcattg	gcacaacaat	aactattatg	cgtttgtagg	tgccgagcat	1680
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catgcagttt	ctcatgtgcg	aagatctcct	ccttctaaac	tgacactaaa	tatgggatat	1920
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aagacaccta	tacaaggatc	cccgctggca	cggcatgcct	tcttcttaga	agtgcatgat	2040
actttgtata	ttcatcattt	tggaagagcc	tatatgaact	attcattaga	tgctcgtcgt	2100
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Asn Asp Ala Asp Ala Ala His Tyr Gly Tyr Gln Gly Ser Trp Ser Ala
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Asp Trp Thr Lys Pro Pro Leu Ala Pro Asp Ala Lys Gly Met Val Pro
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Pro Asn Thr Asn Asn Thr Leu Tyr Leu Thr Trp Arg Pro Ala Ser Asn
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                                          380
Tyr Gly Glu Tyr Arg Leu Asp Pro Gln Arg Lys Gly Glu Leu Val Pro
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Asn Ser Leu Trp Val Ala Gly Ser Ala Leu Arg Thr Phe Thr Asn Gly
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                                  410
Leu Lys Glu His Tyr Val Ser Arg Asp Val Gly Phe Val Ala Ser Leu
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His Ala Leu Gly Asp Tyr Ile Leu Asn Tyr Thr Gln Asp Asp Asp
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Gly Phe Leu Ala Arg Tyr Gly Gly Phe Gln Ala Thr Ala Ala Ser His
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Tyr Glu Asn Gly Ser Ile Phe Gly Val Ala Phe Gly Gln Leu Tyr Gly
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Gln Thr Lys Ser Arg Met Tyr Tyr Ser Lys Asp Ala Gly Asn Met Thr
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                                          540
Cys His Trp His Asn Asn Tyr Tyr Ala Phe Val Gly Ala Glu His
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Asn Phe Leu Glu Tyr Cys Ile Pro Thr Arg Gln Leu Ala Arg Asp Tyr
                                  570
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Glu Leu Thr Gly Phe Met Arg Phe Glu Met Ala Gly Gly Trp Ser Ser
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Ser Thr Arg Glu Thr Gly Ser Leu Thr Arg Tyr Phe Ala Arg Gly Ser
                          600
Gly His Asn Met Ser Leu Pro Ile Gly Ile Val Ala His Ala Val Ser
                      615
                                          620
His Val Arg Arg Ser Pro Pro Ser Lys Leu Thr Leu Asn Met Gly Tyr
                 630
                                      635
Arg Pro Asp Ile Trp Arg Val Thr Pro His Cys Asn Met Glu Ile Ile
              645
                                  650
Ala Asn Gly Val Lys Thr Pro Ile Gln Gly Ser Pro Leu Ala Arg His
                              665
Ala Phe Phe Leu Glu Val His Asp Thr Leu Tyr Ile His His Phe Gly
                           680
Arg Ala Tyr Met Asn Tyr Ser Leu Asp Ala Arg Arg Arg Gln Thr Ala
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His Phe Val Ser Met Gly Leu Asn Arg Ile Phe
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<211> 38

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<213> Chlymadia trachomatis

<400> 330

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                                                                        120
 accettcata tegggeetae egeetteete geettgggtg ttgtegacaa caacggcaac
                                                                        180
 ggcgcacgag tccaacgcgt ggtcgggagc gctccggcgg caagtctcgg catctccacc
                                                                        240
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                                                                        300
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                                                                        360
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                                                                        420
 ccatcacact ggcggccgct catgaaatgg ctgtcagcta ctgcggtgtt tgctgctgtt
                                                                        480
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                                                                        540
                                                                        600
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 tctacaatct ccctatcagg gattactaaa gcgactttct cctgcaactc tgcagaagtt
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                                                                       1320
 gatggaggag cgatctttgc tgagaaagat gtttctttcg agaatattac atcattaaaa
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             20
                                 25
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                             40
 Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val
                         55
                                             60
 Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
                                                              80
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156

Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser 100 105 Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr 115 120 Leu Ala Glu Gly Pro Pro Ala Glu Phe Cys Arg Tyr Pro Ser His Trp 135 140 Arg Pro Leu Met Lys Trp Leu Ser Ala Thr Ala Val Phe Ala Ala Val 150 155 Leu Pro Ser Val Ser Gly Phe Cys Phe Pro Glu Pro Lys Glu Leu Asn 165 170 Phe Ser Arg Val Glu Thr Ser Ser Ser Thr Thr Phe Thr Glu Thr Ile 185 Gly Glu Ala Gly Ala Glu Tyr Ile Val Ser Gly Asn Ala Ser Phe Thr 200 Lys Phe Thr Asn Ile Pro Thr Thr Asp Thr Thr Thr Pro Thr Asn Ser 220 215 Asn Ser Ser Ser Ser Gly Glu Thr Ala Ser Val Ser Glu Asp Ser 230 235 Asp Ser Thr Thr Thr Pro Asp Pro Lys Gly Gly Ala Phe Tyr 245 250 Asn Ala His Ser Gly Val Leu Ser Phe Met Thr Arg Ser Gly Thr Glu 265 Gly Ser Leu Thr Leu Ser Glu Ile Lys Met Thr Gly Glu Gly Gly Ala 280 Ile Phe Ser Gln Gly Glu Leu Leu Phe Thr Asp Leu Thr Ser Leu Thr 295 300 Ile Gln Asn Asn Leu Ser Gln Leu Ser Gly Gly Ala Ile Phe Gly Gly 310 315 Ser Thr Ile Ser Leu Ser Gly Ile Thr Lys Ala Thr Phe Ser Cys Asn 325 330 Ser Ala Glu Val Pro Ala Pro Val Lys Lys Pro Thr Glu Pro Lys Ala 345 Gln Thr Ala Ser Glu Thr Ser Gly Ser Ser Ser Ser Gly Asn Asp 360 Ser Val Ser Ser Pro Ser Ser Ser Arg Ala Glu Pro Ala Ala Asn 375 Leu Gln Ser His Phe Ile Cys Ala Thr Ala Thr Pro Ala Ala Gln Thr 395 390 Asp Thr Glu Thr Ser Thr Pro Ser His Lys Pro Gly Ser Gly Gly Ala 410 Ile Tyr Ala Lys Gly Asp Leu Thr Ile Ala Asp Ser Gln Glu Val Leu 420 425 Phe Ser Ile Asn Lys Ala Thr Lys Asp Gly Gly Ala Ile Phe Ala Glu 440 Lys Asp Val Ser Phe Glu Asn Ile Thr Ser Leu Lys Val Gln Thr Asn 455 460 Gly Ala Glu Glu Lys Gly Gly Ala Ile Tyr Ala Lys Gly Asp Leu Ser 470 475 Ile Gln Ser Ser Lys Gln Ser Leu Phe Asn Ser Asn Tyr Ser Lys Gln 490 495 485 Gly Gly Gly Ala Leu Tyr Val Glu Gly Gly Ile Asn Phe Gln Asp Leu 500 505 Glu Glu Ile Arg Ile Lys

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<213> Chlymadia trachomatis
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<210> 335
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<213> Chlamydia trachomatis
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<212> DNA
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accettcata teggectae egectteete geetteggtg ttgtegacaa caacgecaac
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gcgcttaacg ggcatcatcc cggtgacgtc atctcggtga cctggcaaac caagtcgggc
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gcgacagatg ttggaggtgg tgcttacgta aaaggaaccc ttacttgtga aaactctcac
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cgtctacaat ttttgaaaaa ctcttccgat aaacaaggtg gaggaatcta cggagaagac
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                                                                    1620
gctaaaaaag ccaagatgte cegeatagae caactgaata tetetgagaa eteegetaca
                                                                    1680
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<211> 585

<212> PRT

<213> Chlamydia trachomatis

<400> 337

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                                        475
Ser Thr Pro Ser Ser Ser Thr Val Ser Ser Leu Thr Leu Leu Ala Ala
                                    490
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Ser Ser Gln Ala Ser Pro Ala Thr Ser Asn Lys Glu Thr Gln Asp Pro
                                505
                                                    510
            500
Asn Ala Asp Thr Asp Leu Leu Ile Asp Tyr Val Val Asp Thr Thr Ile
                           520
                                                525
        515
Ser Lys Asn Thr Ala Lys Lys Gly Gly Gly Ile Tyr Ala Lys Lys Ala
    530
                       535
                                            540
Lys Met Ser Arg Ile Asp Gln Leu Asn Ile Ser Glu Asn Ser Ala Thr
                                       555
                   550
Glu Ile Gly Gly Gly Ile Cys Cys Lys Glu Ser Leu Glu Leu Asp Ala
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Leu Val Ser Leu Ser Val Thr Glu Asn
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accettcata tegggeetae egeetteete ggettgggtg ttgtegacaa caacggcaac
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ggcgcacgag tccaacgcgt ggtcgggagc gctccggcgg caagtctcgg catctccacc
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                                                                     960
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gttacattga attgtcctgc gacattctct aacaatacag cctctatagc tacaccgaag
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1260

1320

1380

1440

1500

1560

1620

1680

1740

1800

1860

1920

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aatagcatta cagaaaaaat tactttagaa aacggttctt ttatttttga aagaaaccaa
gctaataaac gtggagcgat ttactctcct agcgtttcca ttaaagggaa taatattacc
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Ile Ala Gly Gln Ile Lys Leu Pro Thr Val His Ile Gly Pro Thr Ala
        35
                            40
Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val
                        55
Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
                    70
                                        75
Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
                                    90
Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser
            100
                                105
                                                    110
Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr
                            120
                                                125
Leu Ala Glu Gly Pro Pro Ala Glu Phe Cys Arg Tyr Pro Ser His Trp
                        135
                                            140
Arg Pro Leu Asp Gln Leu Asn Ile Ser Glu Asn Ser Ala Thr Glu Ile
145
                    150
                                        155
Gly Gly Gly Ile Cys Cys Lys Glu Ser Leu Glu Leu Asp Ala Leu Val
                                    170
                165
Ser Leu Ser Val Thr Glu Asn Leu Val Gly Lys Glu Gly Gly Gly Leu
            180
                                185
                                                    190
His Ala Lys Thr Val Asn Ile Ser Asn Leu Lys Ser Gly Phe Ser Phe
                            200
                                                205
Ser Asn Asn Lys Ala Asn Ser Ser Ser Thr Gly Val Ala Thr Thr Ala
                        215
                                            220
Ser Ala Pro Ala Ala Ala Ala Ser Leu Gln Ala Ala Ala Ala Ala
                    230
                                        235
Ala Pro Ser Ser Pro Ala Thr Pro Thr Tyr Ser Gly Val Val Gly Gly
                245
                                    250
Ala Ile Tyr Gly Glu Lys Val Thr Phe Ser Gln Cys Ser Gly Thr Cys
                                265
                                                    270
Gln Phe Ser Gly Asn Gln Ala Ile Asp Asn Asn Pro Ser Gln Ser Ser
                            280
Leu Asn Val Gln Gly Gly Ala Ile Tyr Ala Lys Thr Ser Leu Ser Ile
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295
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Tyr Ser Pro Thr Val Thr Leu Asn Cys Pro Ala Thr Phe Ser Asn Asn
         340 345 350
Thr Ala Ser Ile Ala Thr Pro Lys Thr Ser Ser Glu Asp Gly Ser Ser
  355 360 · 365
Gly Asn Ser Ile Lys Asp Thr Ile Gly Gly Ala Ile Ala Gly Thr Ala
   370 375
"Ile Thr Leu Ser Gly Val Ser Arg Phe Ser Gly Asn Thr Ala Asp Leu
385 390 395 400
Gly Ala Ala Ile Gly Thr Leu Ala Asn Ala Asn Thr Pro Ser Ala Thr
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Thr Ser Thr His Asp Gly Ser Ala Ile Tyr Phe Thr Lys Asp Ala Thr
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Ile Glu Ser Leu Gly Ser Val Leu Phe Thr Gly Asn Asn Val Thr Ala
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Thr Gln Ala Ser Ser Ala Thr Ser Gly Gln Asn Thr Asn Thr Ala Asn
       500 505
Tyr Gly Ala Ala Ile Phe Gly Asp Pro Gly Thr Thr Gln Ser Ser Gln
 515 520
Thr Asp Ala Ile Leu Thr Leu Leu Ala Ser Ser Gly Asn Ile Thr Phe
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Ser Asn Asn Ser Leu Gln Asn Asn Gln Gly Asp Thr Pro Ala Ser Lys
545 550 555 560
Phe Cys Ser Ile Ala Gly Tyr Val Lys Leu Ser Leu Gln Ala Ala Lys
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Gly Lys Thr Ile Ser Phe Phe Asp Cys Val His Thr Ser Thr Lys Lys
      580 585 590
Thr Gly Ser Thr Gln Asn Val Tyr Glu Thr Leu Asp Ile Asn Lys Glu
      595 600 605
Glu Asn Ser Asn Pro Tyr Thr Gly Thr Ile Val Phe Ser Ser Glu Leu
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  610 615
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36

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<212> DNA

<213> Chlamydia trachomatis

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	Val	Thr	Trp 115	Gln	Thr	Lys	Ser	Gly 120	Gly	Thr	Arg	Thr	Gly 125	Asn	Val	Thr
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			275			Pro		280					285			
	_	290				Ile	295			_		300	_			
	305		_		_	Leu 310		•			315					320
					325	Val				330					335	
				340		Met	_		345					350		
			355			Ala	_	360			_		365		-	
		370				Asp	375					380				
	385					11e 390			_		395					400
					405	Phe				410					415	
				420		Phe			425		_			430		
			435	_	_	Ser	_	440					445	_		-
		450				Ile	455					460				
	465		-			Lys 470	_				475			Ī.	_	480
					485	Ala				490	_	-			495	
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Cys Ala Tyr Arg Asn Leu Ser Leu Pro Val Gly Cys Ala Val Glu Gly
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660

720

780

840

900

960

1020

1080

1140

1200

1260

1320

1380

1440 1464

WO 01/40474 PCT/US00/32919

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Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val
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Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
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Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
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Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser
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Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr
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                           120
Leu Ala Glu Gly Pro Pro Ala Glu Phe Cys Arg Tyr Pro Ser His Trp
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                                           140
Arg Pro Leu Met Lys Phe Met Ser Ala Thr Ala Val Phe Ala Ala Val
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                   150
Leu Ser Ser Val Thr Glu Ala Ser Ser Ile Gln Asp Gln Ile Lys Asn
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Thr Asp Cys Asn Val Ser Lys Val Gly Tyr Ser Thr Ser Gln Ala Phe
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Val Ser Phe Tyr Asp Phe Ser Thr Ser Ser Gly Leu Pro Arg Lys His
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Leu Ser Ser Ser Glu Ala Ser Pro Thr Thr Glu Gly Val Ser Ser
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Ser Ser Ser Gly Glu Asn Thr Glu Asn Ser Gln Asp Ser Ala Pro Ser
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Ser Gly Glu Thr Asp Lys Lys Thr Glu Glu Glu Leu Asp Asn Gly Gly
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Ile Ile Tyr Ala Arg Glu Lys Leu Thr Ile Ser Glu Ser Gln Asp Ser

285

280

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Glu Gly Glu Val Ile Phe Asp His Arg Val Ala Leu Lys Asn Gly Gly
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Leu Val Glu Val Asn Ile Ser Val Glu Lys Gly Gly Ser Val Tyr Ala
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Lys Glu Arg Val Ser Leu Glu Asn Val Thr Glu Ala Thr Phe Ser Ser
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Asn Gly Gly Glu Gln Gly Gly Gly Ile Tyr Ser Glu Gln Asp Met
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                        375
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Leu Ile Ser Asp Cys Asn Asn Val His Phe Gln Gly Asn Ala Ala Gly
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Ala Thr Ala Val Lys Gln Cys Leu Asp Glu Glu Met Ile Val Leu Leu
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                                    410
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Thr Glu Cys Val Asp Ser Leu Ser Glu Asp Thr Leu Asp Ser Thr Pro
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Glu Thr Glu Gln Thr Lys Ser Asn Gly Asn Gln Asp Gly Ser Ser Glu
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Thr Lys Asp Thr Gln Val Ser Glu Ser Pro Glu Ser Thr Pro Ser Pro
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39

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Pro Glu Val Val Ala Ser Ala Lys Ile Asn Arg Phe Phe Ala Ser Thr
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Asp Gln Thr Glu Thr Ser Asp Thr Asn Ser Asp Ile Asp Val Ser Ile
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Tyr Asn Ser Ala Ala Lys Glu Gly Gly Val Ile His Ser Lys Thr Val
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Val Glu Gly Glu Glu Ser Thr Ala Thr Glu Asn Pro Asn Ser Asn Thr
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Ala Asp Thr Gly Thr Gly Val Val Asn Asn Glu Ser Gln Asp Thr Ser
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Asp Thr Gly Asn Ala Glu Ser Gly Glu Gln Leu Gln Asp Ser Thr Gln
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Ser Asn Glu Glu Asn Thr Leu Pro Asn Ser Ser Ile Asp Gln Ser Asn
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The same of the sa

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55
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 Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
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 Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
 Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser
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 Lys Ile Glu Asn Phe Ser Gly Gln Gly Ile Phe Ser Gly Asn Lys Ala
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 Ile Phe Gly Gln Ile Ala Ser Ser Asn Gly Ser Gln Thr Asp Asn Leu
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Ile	Ala	Gly	qeA	Val 565	Lys	Leu	Thr	Met	Gln 570	Ala	Ala	Lys	Gly	Lys 575	Thr
Ile	Ser	Phe	Phe 580	Asp	Ala	Ile	Arg	Thr 585	Ser	Thr	Lys	Lys	Thr 590	Gly	Thr
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